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by

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1.0 ACKNOWLEDGEMENTS

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2.0 INTRODUCTION

This report, covering the period from March 1966 to March 1967, deals with a study of planaria orientation in the diminished magnetic field and a continuation of the development of multi-channel, implantable telemeters for biological research.

It was of particular interest to have the opportunity to carry out research on planarian orientation in the ambient (normal earth's) field and the "null" field, particularly since "null" field studies have not been previously reported. In this study we have not attempted to repeat reported work but rather to apply fundamental MET philosophy in a limited way and within certain economic constraints. With this philosophy in mind, could an experiment be planned in such a way as to eliminate all stimuli but those due to magnetic origin and yield statistically significant results within the boundary conditions?

We have learned much in the course of the work described in Section 3.0 of this report. The results challenge us to further investigation.

The design aspects and statistical techniques have been presented in sufficient detail for other interested workers to repeat the work or modify it if desired.

Lastly, our results lead to conclusions which, we hope, will encourage the determination of the magnetic "threshold" required to produce an orientation effect in the same or other biological subjects.

Section 4.0 of this report continues the description of the work in the development of multi-channel long-life telemetric implants.

3.0 MAGNETIC RESPONSE OF PLANARIA

3.1 INTRODUCTORY DISCUSSION

3.1.1 MAGNETIC EFFECTS ON BIOLOGICAL SYSTEMS

There is an ever-increasing amount of literature pertaining to the influence of homogeneous magnetic fields upon biological systems. The biological systems that have been studied range from the simplest to the most advanced plants and animals. The magnetic conditions that have been applied to these various systems vary, but can be divided roughly into diminished, ambient (normal) and increased field strengths. The biological phenomena that have been most studied under these magnetic conditions include cell growth, plant growth and orientation, and especially animal orientation (navigation).

Many different types of cells have been the subject of growth studies under various magnetic conditions, for example, Hendrick (in Barnothy) (1) applied a homogeneous field of 14,000 Oe to three bacteria. Continuous exposure to this field strength inhibited the growth of one, but not the other two. Halpern and Konikoff (1964) (2) studied the influence of various magnetic field strengths on the growth of two strains of the green algae, *Chlorella pyrenoidosa*. In a 750 gauss field, both strains had inhibited growth. At 1,000 gauss, one strain showed no effect, while the other had slight inhibition. At 4,000 gauss, both strains had enhanced growth.

Plant growth has been shown to be influenced by homogeneous magnetic fields in two ways: Mericle, et al (in Barnothy) (1), for example, reported a statistically significant increase in growth in barley seedling roots and shoots in a high (~ 1200 Oe) field; Halpern reported increased percentage of seed germination in low fields (3) In non-homogeneous fields, plants have been reported to display a tactic response, Audus and Whish (in Barnothy) (1).

The single phenomenon that has received most interest is animal orientation (or navigation). Work has been done on *Paramecium*, *Volvox*, *Dugesia dorotocephala*, *Nassarius obsoletus*, and birds. Most work tends to indicate that these organisms orient with respect to magnetic fields.

The system that has been most intensively investigated is the planaria, *Dugesia dorotocephala* by Brown. Brown's work is discussed in subsequent sections.

3.1.2 REASONS FOR STUDY

Both on earth in a natural environment and in regions of a highly developed applied technology man is exposed to magnetic fields. In general these magnetic fields are within the earth's magnetic field with its known limits, or man-made magnetic fields not greatly larger or smaller than this field. In space, or on the moon's surface man will be exposed to magnetic fields several orders of magnitude smaller than the earth's field. Interest in the performance of man in a region of very low magnetic field is thus pertinent. As a first step towards determining the performance of man in low fields, determining the performance of simple animals in low fields was considered reasonable.

Planaria exhibit a large number of orientative or navigational responses. Among these is a response to magnetic fields. In the present study we were interested in the response to a magnetic field which was considerably reduced from ambient.

The study of the difference in the ability to navigate in the ambient field and in greatly reduced magnetic field under a constant light gradient was felt to be of significance to this general problem.

3.1.3 SPECIFIC MAGNETIC PROBLEM

3.1.3.1 Planarian Orientation (Navigation)

In this experiment as in any experiment, we must choose two hypotheses, a main hypothesis and an alternate one. In this case, we have formulated our main hypothesis as a null hypothesis. It can be stated as: There is no difference between the direction taken by the worms when they are exposed to earth's ambient magnetic field and the direction taken when this field is considerably reduced by shielding. At all times the worms are forced to travel in one of four directions measured clockwise with respect to the earth's N-magnetic vector: 0°, 90°, 180°, and 270° (i.e., N, E, S, W).

All other environmental conditions remain as nearly constant as possible.

Other null hypotheses are possible with slightly changed experimental conditions. The earth's field may be augmented or reduced. The turning of the worms may be measured under different light conditions and gradients. The difference in turning from the average turning may be considered. In this experiment it was felt that the difference angle obtained under shielded and non-shielded conditions was the best and most straightforward way of dealing with the question of whether or not there was a magnetic "guiding" effect.

There are in general an infinite number of hypotheses which are the alternative of any null hypothesis. The most general, and at the beginning of an investigation, the safest alternative hypothesis if one wants to have no more than two choices is to have the alternate hypothesis as the opposite of the null hypothesis. Thus, the alternate hypothesis in this case will be that there is a magnetic effect of some kind on the navigation of the worms, but that its magnitude and/or direction is not specified. In the absence of other information under the experimental conditions imposed in this experiment no other hypothesis can be reasonably made.

3.1.3.2 Possible Approaches to the Problem

Brown (4,5,6,7,8,9) has made extensive studies of the effect of a magnetic field on planaria under several specific conditions. In summary, his experiments show that worms, when forced to travel at an angle to a magnetic field vector, deviate from this angle by a few degrees depending upon the intensity and direction of the magnetic field. His angular measurements also were correlated to the phase of the moon which caused a cyclical deviation from the average angle. Other cyclical (such as yearly) effects were also observed. In general, Brown's analysis determined the turning toward or away from a line, towards which the worms were forced by their negative phototactic response, located approximately 65°, 155°, 245°, and 335° clockwise from the N magnetic vector. The turning was measured as the difference angle between the average for one direction and the average for the four directions. In our experiment the control was essentially the null field condition achieved in the shield. The angular difference between this angle and the angle obtained under identical conditions, but in the earth's ambient magnetic field, constituted the variable angle in which we were interested.

The difference or turning angles determined by Brown showed a definite but small difference towards a north-south line (i.e., the north magnetic vector without regard for the sense of that vector). The effect was small (on the order of one degree) and for statistical significance required the average of several thousand path determinations. In some experiments by Brown the earth's field was augmented or decreased by means of magnets of known magnetic field strength. Our results are also quite small angles and similarly require a large number of measurements for a significant effect.

In preliminary work with the worms various methods of guiding them were tried. The worms have certain traits which do not permit just any experimental arrangement to be used. For example, a worm will move on an unobstructed horizontal plane until it reaches a wall; it will then follow this wall to the wall's end. If the wall makes a sharp bend at 90° or greater, the worm, about half the time, will separate from the wall at an angle about 45°. About half the time it will make the sharp bend and continue along the wall. Various walls, wall angles, slopes, channels both V shaped and U shaped were tried. Corrals with an opening oriented in the desired direction, cylinders with holes in them and other geometric configurations of this type were tried but did not prove satisfactory. The worms did not consistently choose to exit the guiding device in a regular fashion. In several preliminary experiments the worm was placed inside a small ring about $\frac{1}{4}$" in diameter at the center of a polar grid and allowed to choose his own initial direction under the influence of the light gradient. This did not prove satisfactory because a fairly large proportion of the worms chose to travel towards the light at varying degrees from right angles to directly towards the light. Because of this, in a limited number of measurements the average direction had an enormous variance. In addition, because of the large angles involved it was necessary to use a vector addition of the angles, which added greatly to the work of processing the data. Since the variation was so large, it was felt that no useful information could be obtained fron these data even though this experimental procedure was preferred because the worm was given a free choice of direction. There were additional problems with this procedure. In approximately 10% to 25% of the starts the worm would not leave the ring. Also, it was observed that the light gradient was not uniform due to the shadow of the ring itself. For these reasons, this procedure was discarded and the final procedure was worked out and adopted.

In the present study a different approach was felt to be more direct. This approach was based on removing the ambient (earth's) magnetic field by means of a magnetic shield fabricated from mu-metal, a material of very high magnetic permeability. Each worm was initially oriented and directed (by a light gradient) towards each one of the compass directions with or without the magnetic shield. Eight different conditions were thus imposed upon the worm. Each worm experienced these conditions in a different random sequence. This is different in several respects from the procedure used elsewhere. One, our worms were oriented and directed in the same direction. Brown's worms were forced to turn approximately 25° counterclockwise (as seem from above) from their initial orientation. Two, in most of the work reported the control paths were either the earth's field or an average of the paths taken in four directions at 90° from each other. In our experiments the control paths were considered to be those in the reduced (or null) field condition. Three, in some of the other studies the directing light was extinguished as soon as the worm started its travel. In this experiment, a single directing light was on at all times. Four, in this experiment, the directing light was in line with or at multiples of 90° to the earth's N-magnetic vector.

One other aspect which we considered of importance was the use of a clean dish for each path or traverse of a worm. It was observed during preliminary work that a definite though faint slime trail was left by a worm as it travelled across the bottom of a dish. This trail left by one worm could very possibly influence the path taken by a subsequent worm. Whether this would result in an avoidance or an enforcement was not determined, but it was felt to be an influence which might confound the results. Therefore identical plastic petri dishes were used and were thoroughly cleaned after each use. Every worm path angle was taken on a clean dish.

3.2 MATERIALS AND METHODS

3.2.1 BIOLOGICAL MATERIALS

The maintenance of a healthy colony of planaria for some months was essential to the success of this experiment. Early attempts were marred by death of the worms in a few weeks. Common brown planaria, Dugesia tigrina (= P. maculata) purchased from the Carolina Biological Supply Company (CBSC) and cared for as recommended by them did not thrive. It was found necessary to clean their dishes at least three times per week to keep them healthy. The food recommended for the planaria supplied was egg yolk, and while they ate this without hesitation, they did poorly. Beef liver sliced thin was found to be a much better food. The worms were allowed to feed for at least two hours every Friday and placed in clean dishes immediately after feeding. In early January the planaria started depositing cocoons which were removed from their dishes during cleaning and maintained separately. In a few weeks these cocoons hatched and several hairlike worms were obtained from each. Cocoon production was greatest during February and has continued into May. Four dishes of approximately 25 worms each have been maintained since late November of last year to the present. Because of natural fragmentation and regeneration into normal-looking adults the population has remained approximately constant even with a small percentage dying off. These were the worms used in the experiments. While a count was not made of the number of cocoons, it is on the order of 100, and at least 150 new worms have been raised from this source. A separate dish of worms obtained as one of the first purchased batches and which shrank both in size and number to about 12 worms has been brought back to health and vigor and now numbers about 45. These worms have also deposited cocoons but not as many as the groups used in the experiments. The dishes used were 150 mm diameter x 75 mm high crystallization dishes filled with about $1\frac{1}{2}$ inches of Carolina Spring water, purchased in 5 gallon jugs from CBSC as needed and covered with cardboard to prevent evaporation. A separate colony of wild planaria (not identified as to species but apparently different) were obtained from Red Clay Creek outside of Philadelphia. It has also been maintained successfully but has not been used in the experiments.

After the experiments were completed an attempt to maintain the planaria in an artificial spring water recommended by Pace for Amoeba proteus (10) was made. A few animals were tested for a few days but were returned to CBSC spring water when it appeared that they would not survive. Boiled, aereated Philadelphia tap water was tried next and has proved (after more than a month's use) to be as good and probably better than the purchased spring water. The planaria were maintained in the environmental chamber where the temperature is held constant to 21 ± 0.3°C or better and 50 ± 3% R. H. The dishes are kept out of direct light. Chamber lighting is turned on automatically at 6 AM and off at 6 PM.

3.2.2 PHYSICAL MATERIALS

The environmental chamber where the experiments were performed is 18 feet long, 8 feet wide and 8 feet high. It is equipped with temperature, humidity and light controls and is outfitted with laboratory work benches along each side as well as a sink. A strong flow of air throughout the chamber keeps all parts at sensibly the same temperature and relative humidity. The temperature has been held at 20 ± 0.3°C and humidity at 50 ± 3% R.H. for over six months. Several temporary interuptions in the power and water allowed the temperature to depart from this value by a few degrees for periods of less than about 2 hours and the humidity to fall as low as 30% during one period of about six hours. These changes were not considered serious and steps have been taken to prevent their re-occurrence.

The experiments were performed in the apparatus shown in Fig. 3.2-1 The lower part containing the light source was simply a 9" x 9" x 4" wooden box upon which the aluminum cylinder stood. Inside the aluminum cylinder was a 4-3/8" high block of rigid plastic foam on which was mounted the polar grid placed between two 1/16" sheets of lucite. The plastic petri dish was centered on this surface. The buttom of the dish was thus halfway between the top and bottom of the shielding cylinder. The inside of the aluminum cylinder was painted dead flat black with 3M Velvet Black* spray paint. Once

*Minnesota Mining and Mfg. Co., Velvet Black No. 101-C10.

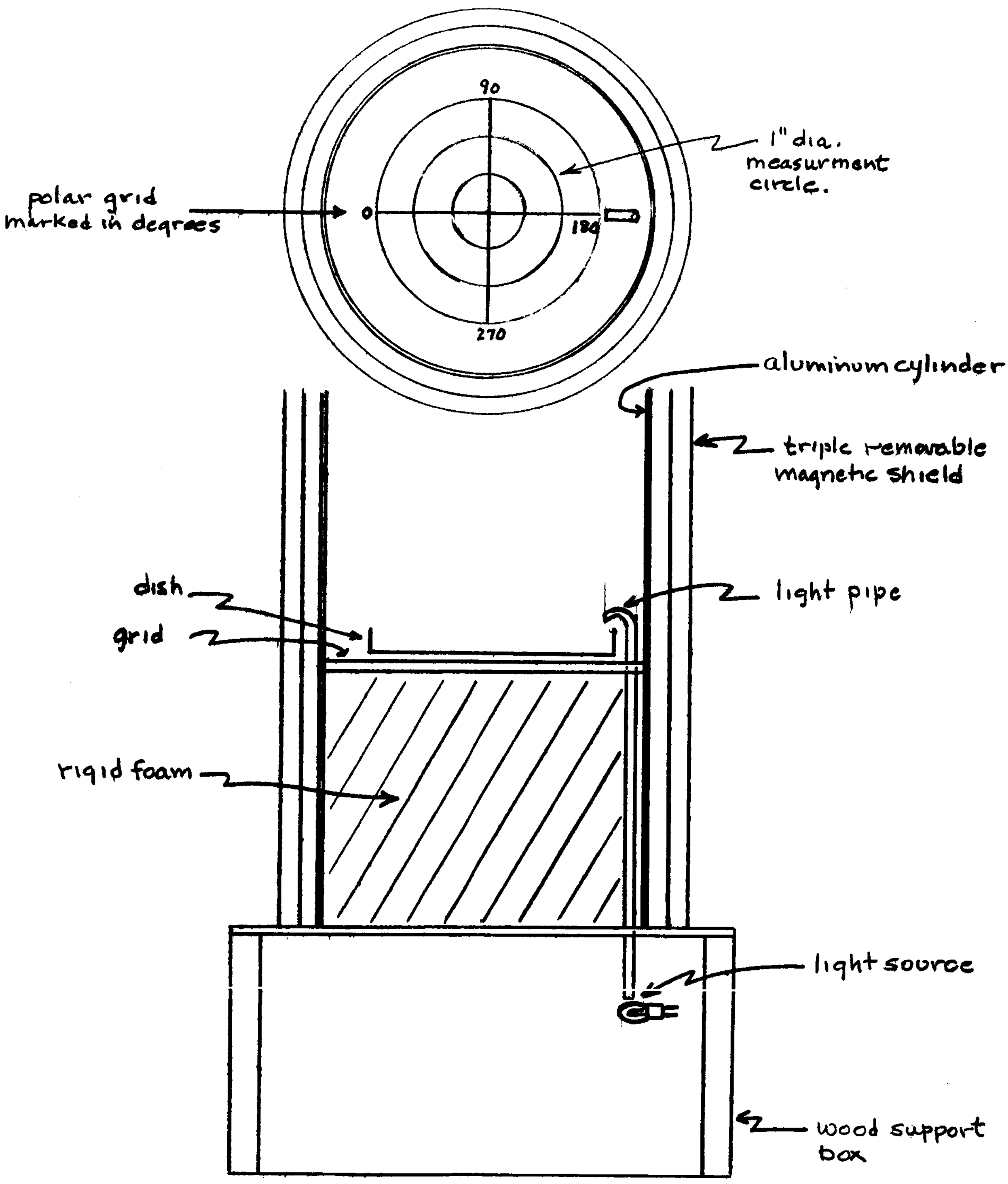


Fig. 3.2-1 - Experiment Apparatus for Determining Effect of Reduced Magnetic Field on Path Taken by Planaria

the cylinder, grid, lamp and light pipe were aligned and positioned they were fastened in place and were not changed for the duration of the 24 days of experiments. The triple mu-metal shield fits snugly around the aluminum cylinder and could be slipped into place without disturbing the rest of the apparatus. The whole box was portable and was rotated on the table top to the desired position against fixed stops.

Fig. 3.2-2 shows the mu-metal shield being placed into position. No ferrous materials were used in the construction of the apparatus with the exception of a small part of the lamp socket, which as far as could be determined did not disturb the field in the measurement area.

A triple mu-metal shield 9" long x 6" inside diameter was used to produce a region of greatly reduced magnetic flux.* This shield is shown in Fig. 3.2-3. Measurements made of the magnetic flux strength in the region where the worm moved both with and without the shield are given in Table 1. A Forster-Hoover magnetometer with a cylindrical probe 2-7/8 long by 1" diameter, Fig. 3.2-4, was used to measure the magnetic flux strength. This instrument has a maximum sensitivity of 0.1 millioersted full scale. In the shielded region of measurement the flux normally fluctuated

*See Final Report F-B2299 Vol. 1, Sec. 3, for complete description and design of this shield. In its present use the end plates were not used.

Fig. 3.2-2 - Shield Being Placed in Position

Fig. 3.2-3 - Observing Path of Worm in Experimental Apparatus

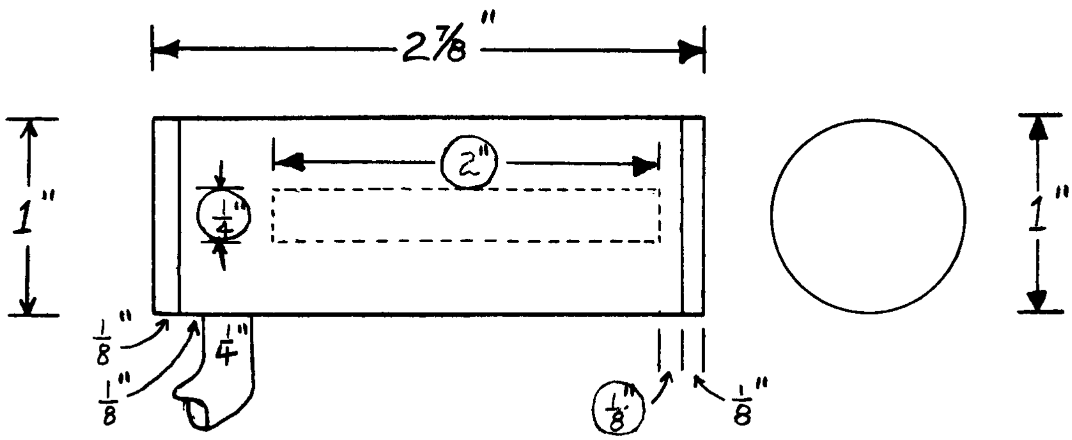


Fig. 3.2-4 - Magnetometer Probe Used With
Forster-Hoover Magnetometer

○ circled dimensions hidden but estimated approximately ± 2 millioersted in the horizontal (N) direction and ± 8 millioersted in the vertical direction without the shield and about ± 0.05 millioersted with the shield in place horizontally and ± 0.5 millioersted in vertical direction. By removing and replacing the probe, differences in the flux were obtained on the order of ± 2 millioersted. These differences and fluctuations were caused for several reasons: The field is not uniform in the cylinder. This is expected since the earth's field hits the shielding cylinder at a steep angle. The field lines through the cylinder are nearly but not exactly parallel to the axis of the cylinder. Measurements made at a large angle to the maximum field are quite sensitive to angular position of the probe. Fig. 3.2-5 shows a diagram with an approximate representation of the lines of flux. And lastly, local (both inside and outside the building) fluctuations in use of power equipment and movement of masses of metal will cause minor fluctuations of this order of magnitude.

The magnitude and directions of the fields when the shield was in place indicate that at the maximum there was a horizontal field in the region where the worm travelled not exceeding 5.3 millioersted and probably not less than 2.3 millioersteds as outside limits to the field, in a northwesterly direction (51 to 52° west of north). This did not vary appreciably for any rotational orientation of the cylinder. This is the field that is termed the "null" field. This represents an attenuation in the horizontal plane of at least 30.7,

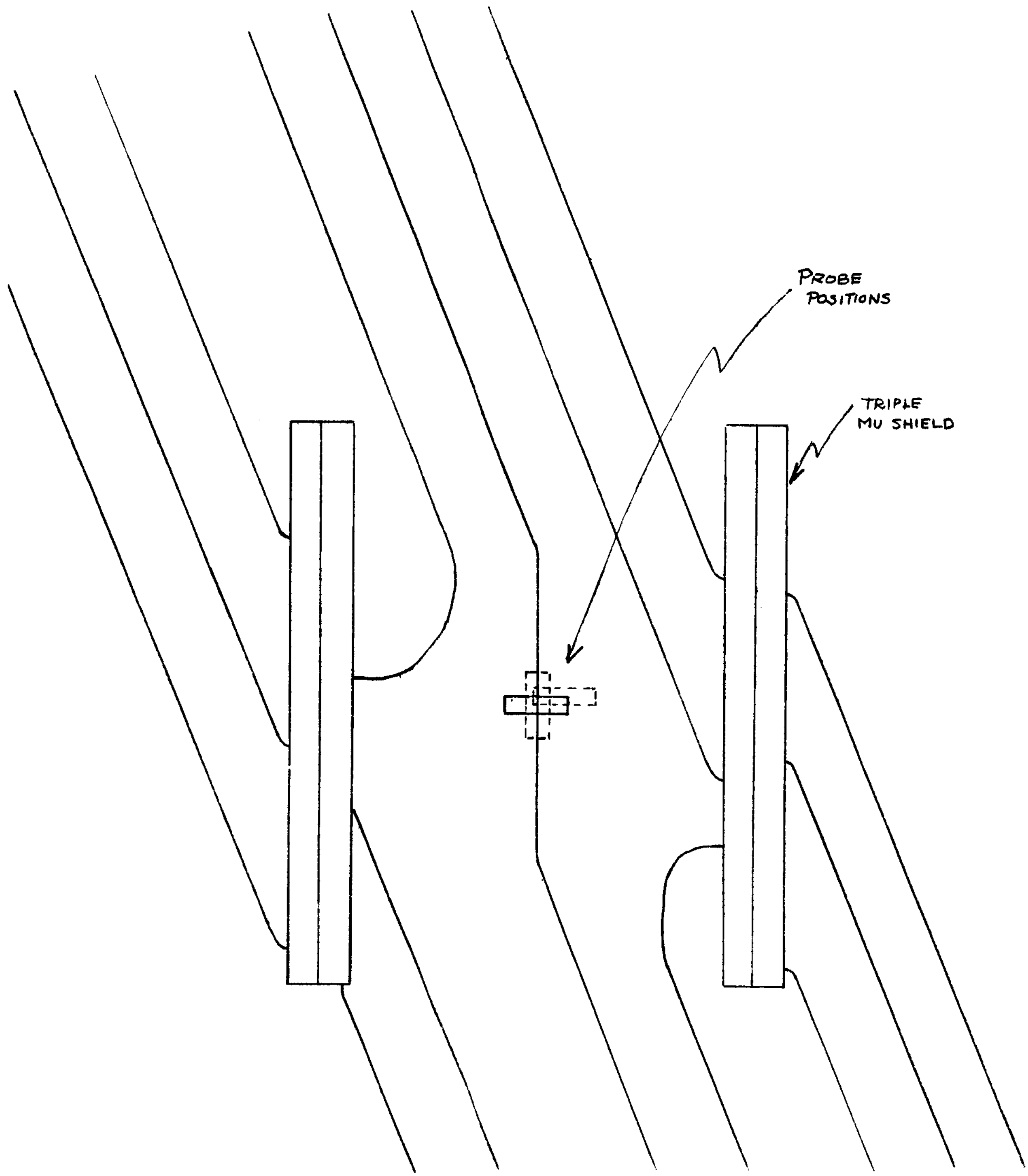


Fig. 3.2-5 - Schematic Flux Distribution Around Triple Shield in Earth's Field

but probably not greater than 66.5. An attenuation of 50 is considered to be most likely in the horizontal plane. In the vertical direction an attenuation between 6.8 and 8.7 was achieved.

TABLE 1

Position	Condition	N	E	S	W
		Direction of Measured Component (Values in Millioersteds)			
Horizontal	No shield	158 ± 5	-	-	-
Horizontal	Shield	1.9 ± .4	⁻2.8 ± 1.1	⁻2.3 ± 1.3	2.4 ± .7
Vertical	No shield	North Down	$640 < H_v < 680$	over the 2" dia. region	
Vertical	Shield	North Down	$78 < H_v < 94$		

Field strengths in millioersteds in region where worms travelled.
+ sign indicates outward from axis of symmetry of the shield
- sign indicates toward axis of symmetry of the shield

Using intermediate figures for the horizontal and vertical components of the earth's field a total magnetic vector of 679 millioersteds at an inclination of 76.3^{o} from the horizontal is determined without the shield. This compares reasonably with a general value of 71^{o} to 72^{o} inclination with horizontal intensity of 180 to 190 millioersteds for Pennsylvania (from the Handbook of Chemistry and Physics). The field at different locations in our laboratory is known to vary in both intensity and direction due to iron in the building structure.

A single light source was used to drive the negatively phototactic worms toward the zero index of the measuring grid. A 7" length of $\frac{1}{4}$" diameter lucite rod with squared and polished ends was bent at one end to form the hook-shaped light pipe as shown in Fig. 3.2-6. This was positioned so that its lower end was within $\frac{1}{4}$" of a small frosted lamp and the other end extended over the tip of the plastic culture dish as shown. Various arrangements were tried before this design was settled on. With other arrangements the light formed irregular and non-uniform patterns due to reflections from and refractions in both the dish and the water in the dish. This design gave the most uniform light pattern and distribution which we could find. The distribution of light is shown in Fig. 3.2-7. It was measured with a photovoltaic cell and calibrated meter. Since the photocell was about $1\frac{1}{2}$" in diameter, the readings are averaged over this area and are thus approximate, but are representative of the illumination used. The light pattern was as uniform from left to right as we could reasonably make it. No doubt some slight asymmetry existed, which, however, was not of serious consequence because of the use of difference angles in the treatment of the data. Approximately a 4 to 1 gradient in light intensity existed from the region where the worm was not supposed to go to the region where it was supposed to go and 2 to 1 from where it started to where it ended. The light was kept on at all times before, during, and after the worm transit. Most of the

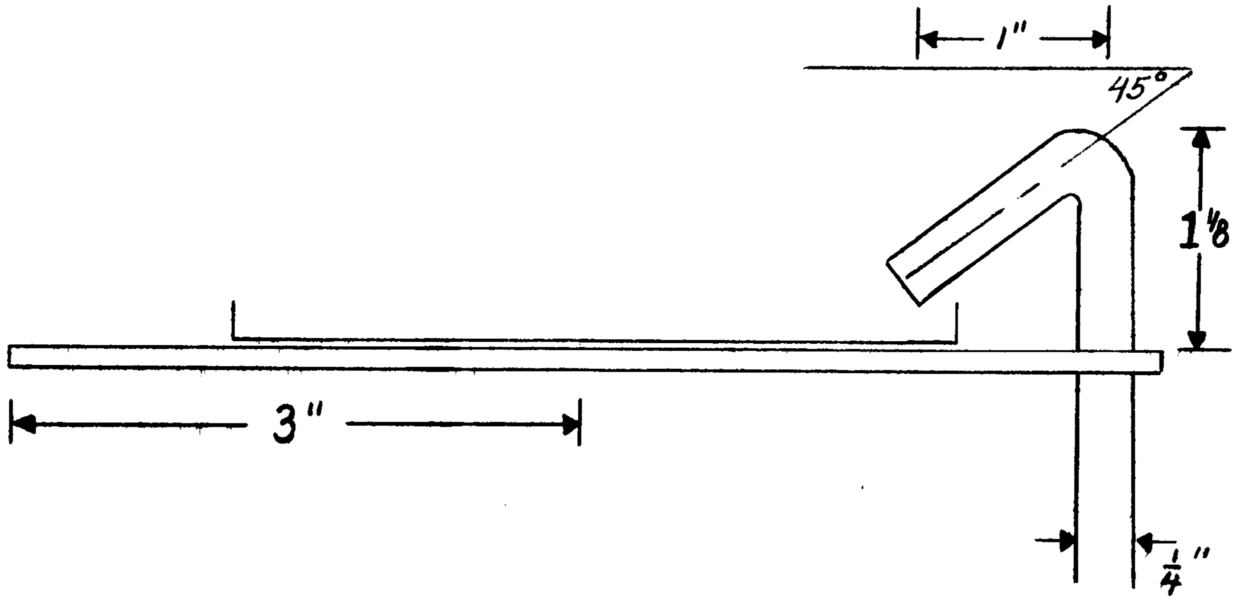
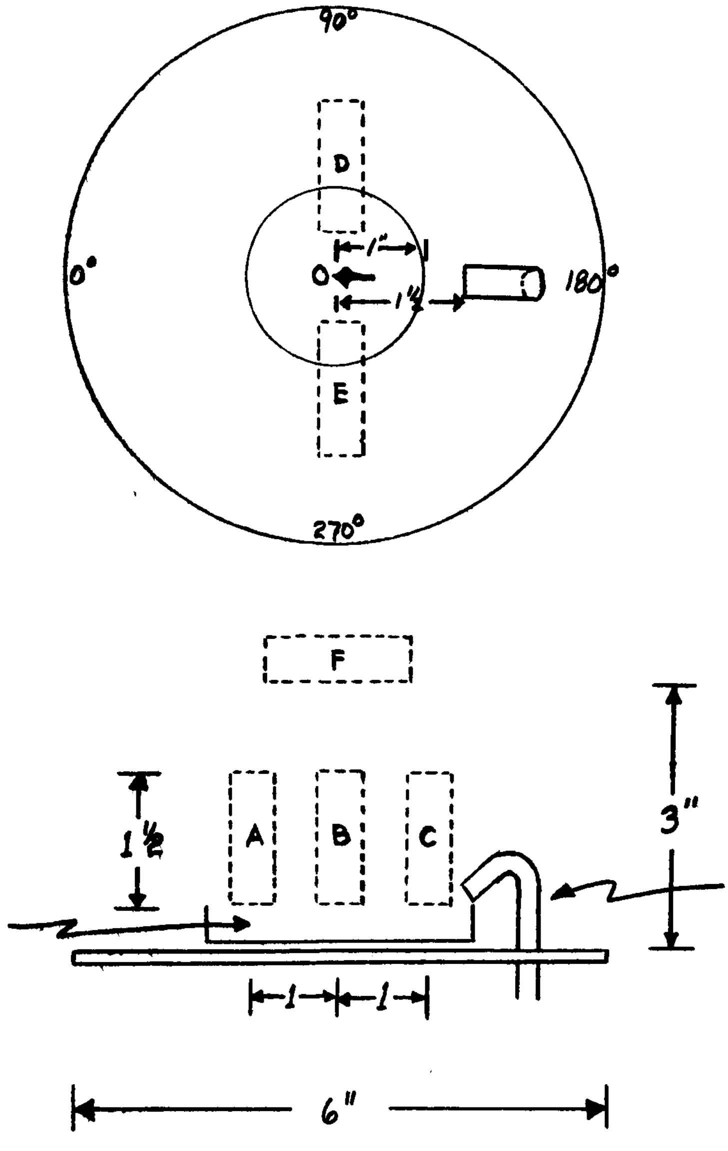


Fig. 3.2-6 - Dimensions of Light Pipe and Position Relative to Plastic Dish



POSITION OF P.C.	A	B	C	D	E	F
LIGHT INTENSITY FOOT-CANDLES	0.6	1.0	2.4	0.6	0.6	0.4

Fig. 3.2-7 - Diagram and Table Showing Photocell Positions and Light Intensity Used to Drive Worm From Center Position Towards 0° Index

worms went directly away from the center where they were initially positioned, crossing the 1" circle where their angle was measured. Out of over 3456 paths measured, only a few (less than 10) were rejected because they were too slow, turned too far (greater than 70° right or left) took a zig-zag path, swam on the surface or stopped completely.

3.2.3 EXPERIMENTAL PROCEDURE

The worms were grown in four groups in 150 mm dia. x 75 mm high pyrex crystallization dishes. These four groups were used in rotation so that at least one week elapsed between the use of any particular dish of worms. Each dish contained 20 to 35 worms, and from these were selected the 18 healthiest looking for a day's run. Worms were fed on Friday after the day's run so that they were never run soon after feeding. The environmental chamber was darkened completely during the experiment except for a small shielded light for recording data and selecting worms. A selected worm was picked up with a large-bore eyedropper and placed in a clean petri dish near the center of the grid. (Fig. 3.2-8) It was gently aligned (by means of the dropper) behind the center and in line with the "0" direction. The use of the smooth-end dropper seemed to be less irritating to the worm than the use of a soft, fine brush. The individual bristles of a brush can create a very high pressure on the worm with very little force exerted by the experimenter because of their extremely small diameter. The worm was observed until

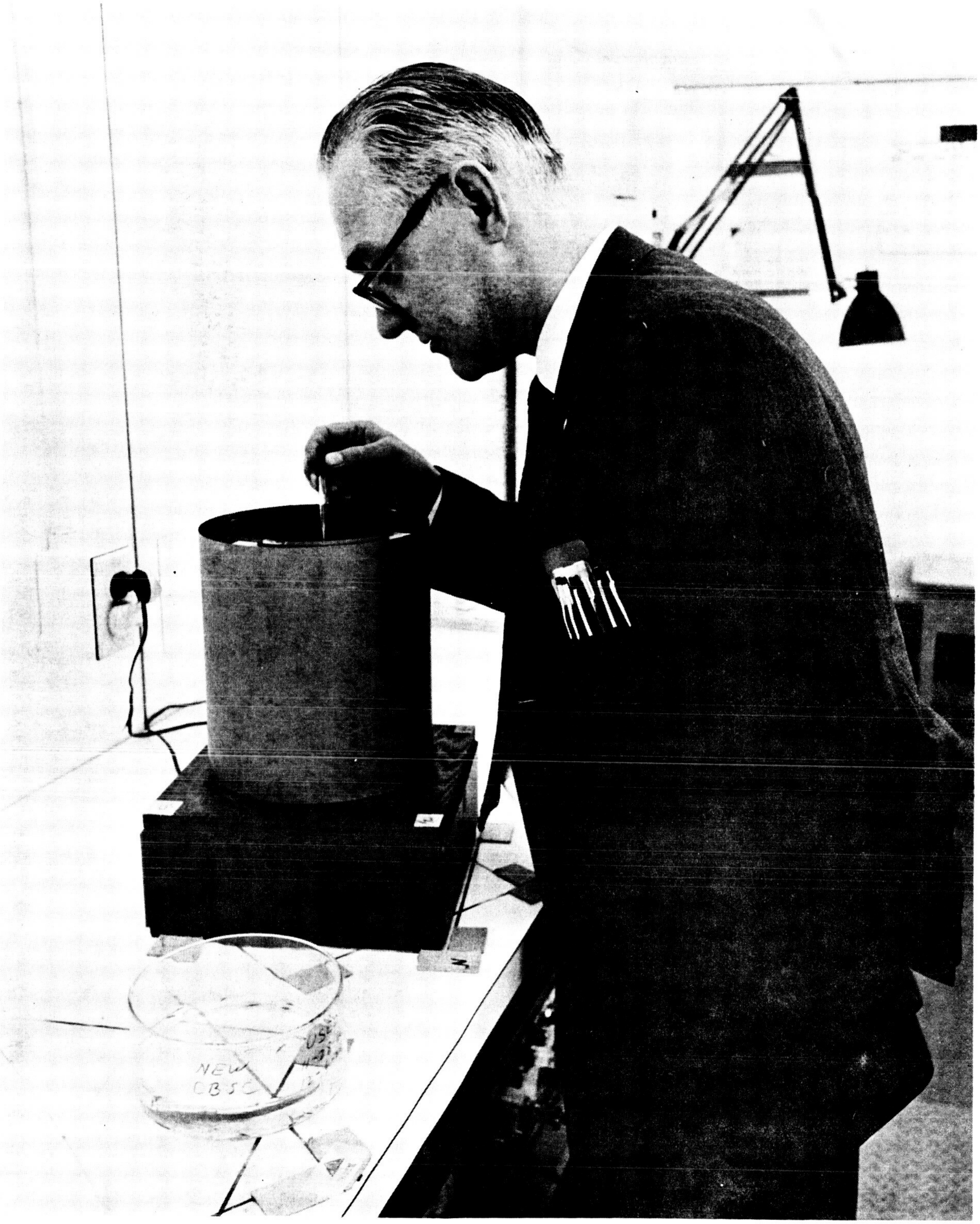


Fig. 3.2-8 - Placing Worm in Position with Pipette

its head just crossed the 1" radius circle, and the intersection at this point determined the measured path angle. By means of 2^{o} graduations at this radius it was possible to estimate the angle to 1^{o}. Other possible angles could have been used at this point. The worm did not usually travel in an exactly straight line on a radius so that the angle of heading between the line down the center of the worm and the "0" direction could have been used as the angle of measurement. This could have been rather difficult to determine but is worth considering for future tests. After a run the worm was picked up from the dish by the dropper, the apparatus turned to the next required heading and the magnetic shielding cylinder put into place or removed as required by the sequence of random numbers. A clean plastic petri dish with 35 cc of spring water was placed into position upon the grid. The worm was returned to the cylinder and positioned as before.

Plastic petri dishes 100 mm diameter x 15 cm high wall were used. After 16 runs the dishes were washed in running water, rubbed with a clean paper towel, rinsed in tap water, shaken dry and refilled with the 35 cc of spring water ready for the next set of 16 runs. The "used" worms were placed in a separate dish so that no worm was utilized for more than one set of 8 runs on any one day. After a day's runs the used and unused worms were combined and kept until their next turn approximately one week later.

3.3 PRESENTATION OF DATA AND METHODS OF ANALYSIS

3.3.1 EXPLANATION OF PROCEDURE

Each day on which an experiment was performed 18 different worms were allowed to run under 8 different conditions. These 8 conditions consisted of each of the four magnetic directions with and without magnetic shielding. An aluminum shield (to exclude light and to provide identical visual conditions for both shielded and unshielded runs) was permanently in place around the apparatus. When shielding was required a triple cylindrical shield of Mu-metal was placed in position surrounding the aluminum shield by slipping it over the top of the permanently-positioned aluminum cylinder. These two conditions are referred to as Al and Mu. In the Al condition the normal ambient earth magnetic field was traversing the area where the worms ran. In the Mu condition the earth's field was reduced to between 2 and 5 millioersteds in the horizontal plane and to between 80 and 90 millioersteds in the vertical direction in the same area. See Table 1. The Mu condition will also be referred to as the "Null" condition and the Al as ambient.

The entire apparatus was placed on a bench and was easily rotated to any desired direction. The direction and sense of the earth's magnetic field were determined in the plane where the worms would run. Stops were placed on the bench so that the apparatus could be quickly and easily aligned so that the light, grid and thus the initial path direction of the worms when aligned would be in one of the four magnetic directions: N, E, S, or W. Magnetic N direction corresponded approximately to geographic north direction. The exact deviation between geographic north and magnetic north could not be determined in the laboratory.

Each worm made 8 trips on any day, designated by the numbers 1 through 8 as shown below:

	N	E	S	W
Al (earth's field)	1	2	3	4
Mu (null field)	5	6	7	8

The order in which these trips were made was determined randomly. Tables of random numbers of the sequence 1 through 8 were prepared before the experiment was performed and were followed exactly.

A typical sequence might be 46218537 followed by the sequence 25137648 for the next worm. Worms were run on 24 different days over a period of $2\frac{1}{2}$ months between the hours of 9 AM and 4 PM. Thus for 8 (conditions) x 18 (worms) x 24 (days) = 3456 paths were observed.

Two different observers were used, "I" and "P". "I" trained "P" so that as nearly as possible their techniques would be the same. As it turned out "I" would complete a run of 18 worms in about one hour less than "P". A complete day's runs required less than 6 hours (between 9 AM and 3 PM). As will be shown later, the variances of the data taken by "P" were larger than those of "I".

3.3.2 ANALYSIS

An analysis of variance (ANOVA) of all the data was performed under three different assumptions for combining the angles. The rules for performing the ANOVA were those given by Winer (11) as developed by Cornfield and Tukey in 1956. The ANOVA in each case showed a main effect which was significant. These main effects in the four directions were then tested using a two-tailed t test. These are explained later. The criterion measure used was the difference angle (Al-Mu) = X, where

"Al" is the observed angle of run for any worm under the ambient condition

"Mu" is the observed angle of run for any worm under the shielded condition

(Al-Mu) is formed as the difference of angle of run for a single worm in any of the four directions.

Figs. 3.3-1 and 3.3-2 show the average angle for each day in each direction for the 24 experiments for the angles measured in the unshielded condition (Al).

Figs. 3.3-3 and 3.3-4 show the average angle for each day in each direction for the 24 experiments for the angles measured in the shielded condition (Mu).

Tables A, B and C in Appendix I give the mean angle in the aluminum cylinder (no shield), mean angle in the mu-metal shielding cylinder and the mean difference angles with their standard errors, each average (of 18 worms) and for 24 experimental days.

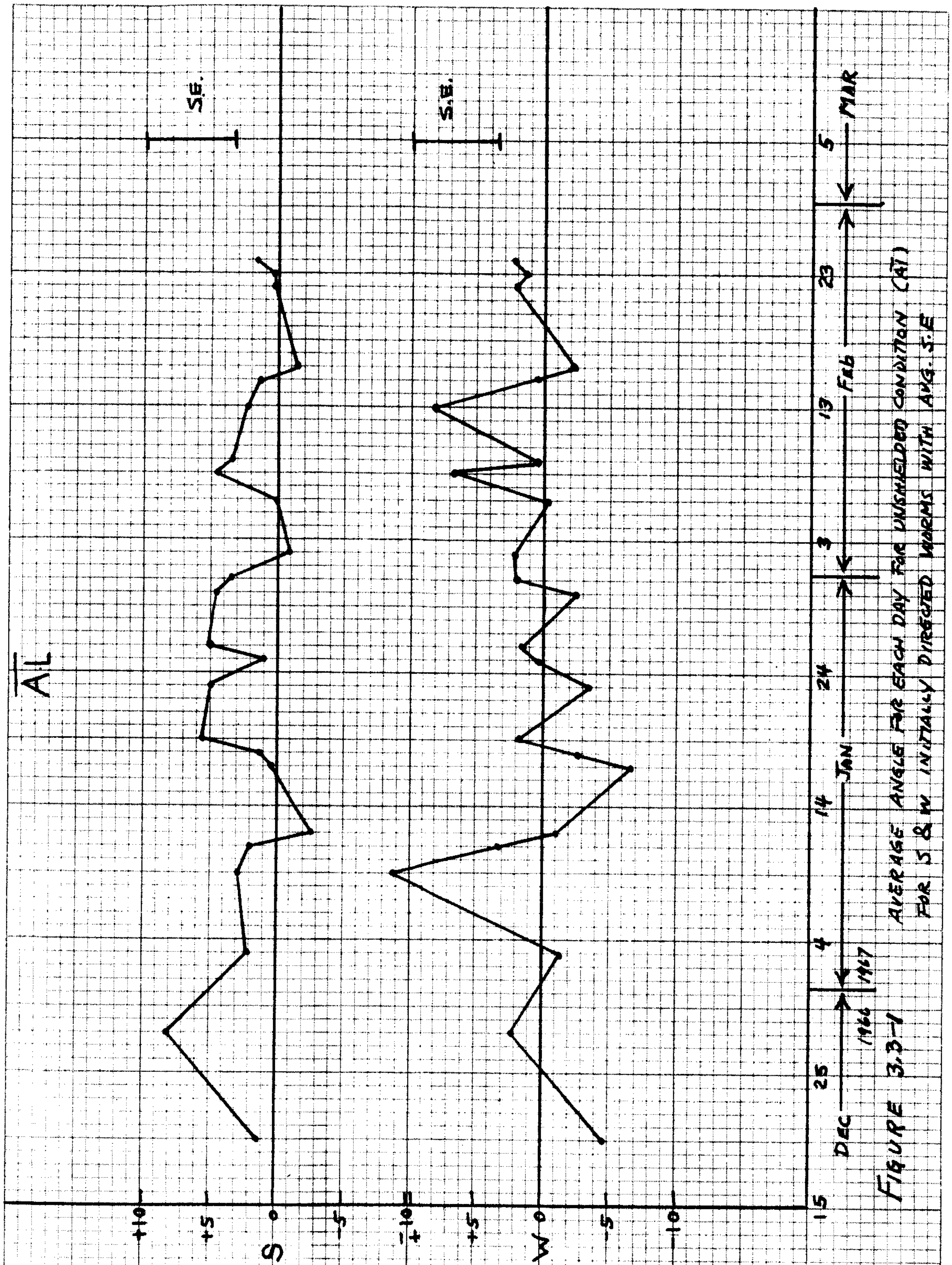


FIGURE 3.3-1 AVERAGE ANGLE FOR EACH DAY FOR UNSHIELDED CONDITION ($\overline{AT}$) FOR S & W INITIALLY DIRECTED WORMS WITH AVG. S.E

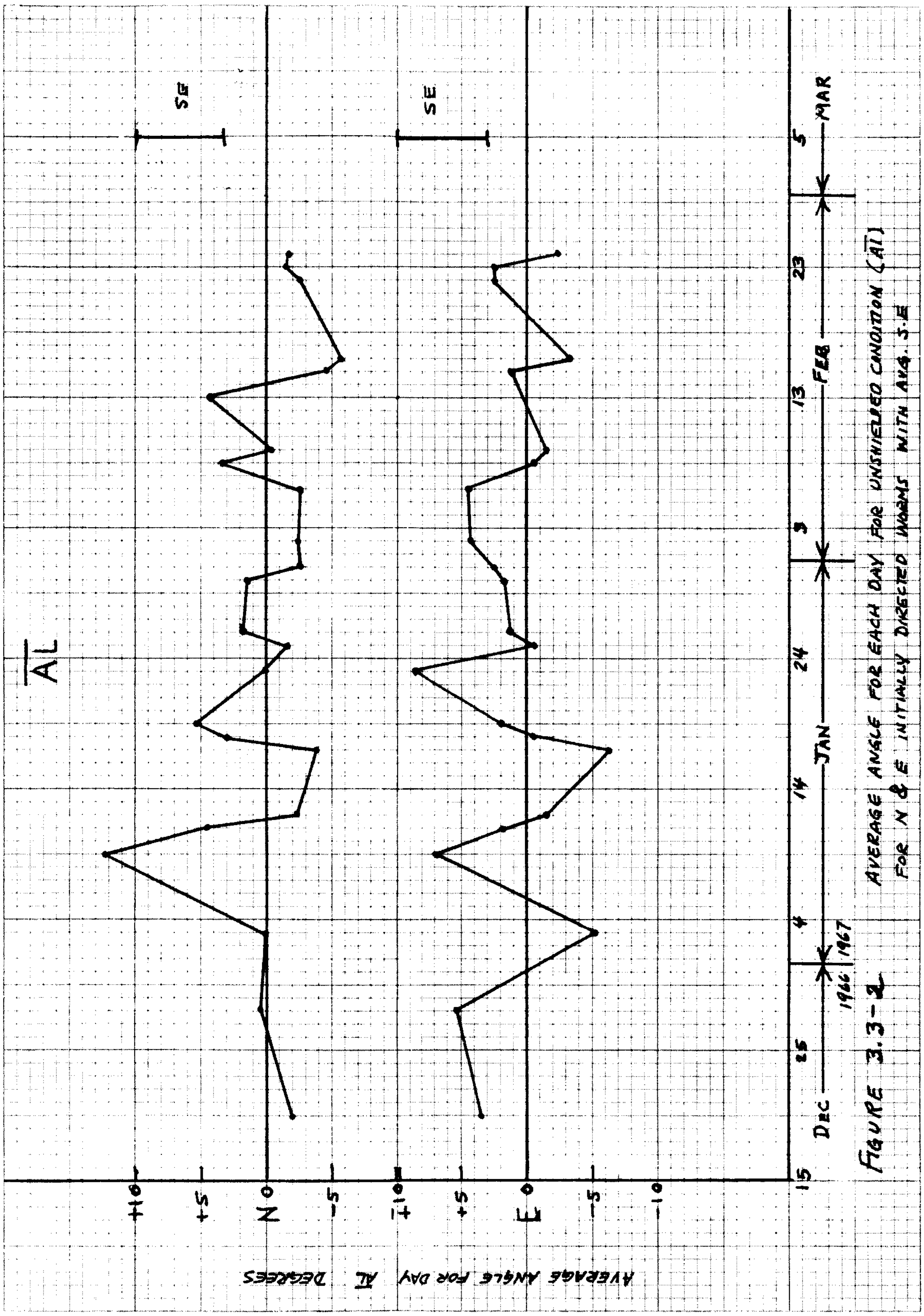


FIGURE 3.3-2 AVERAGE ANGLE FOR EACH DAY FOR UNSHIELDED CONDITION ($\overline{AL}$) FOR N & E INITIALLY DIRECTED WORMS WITH AVG. S.E

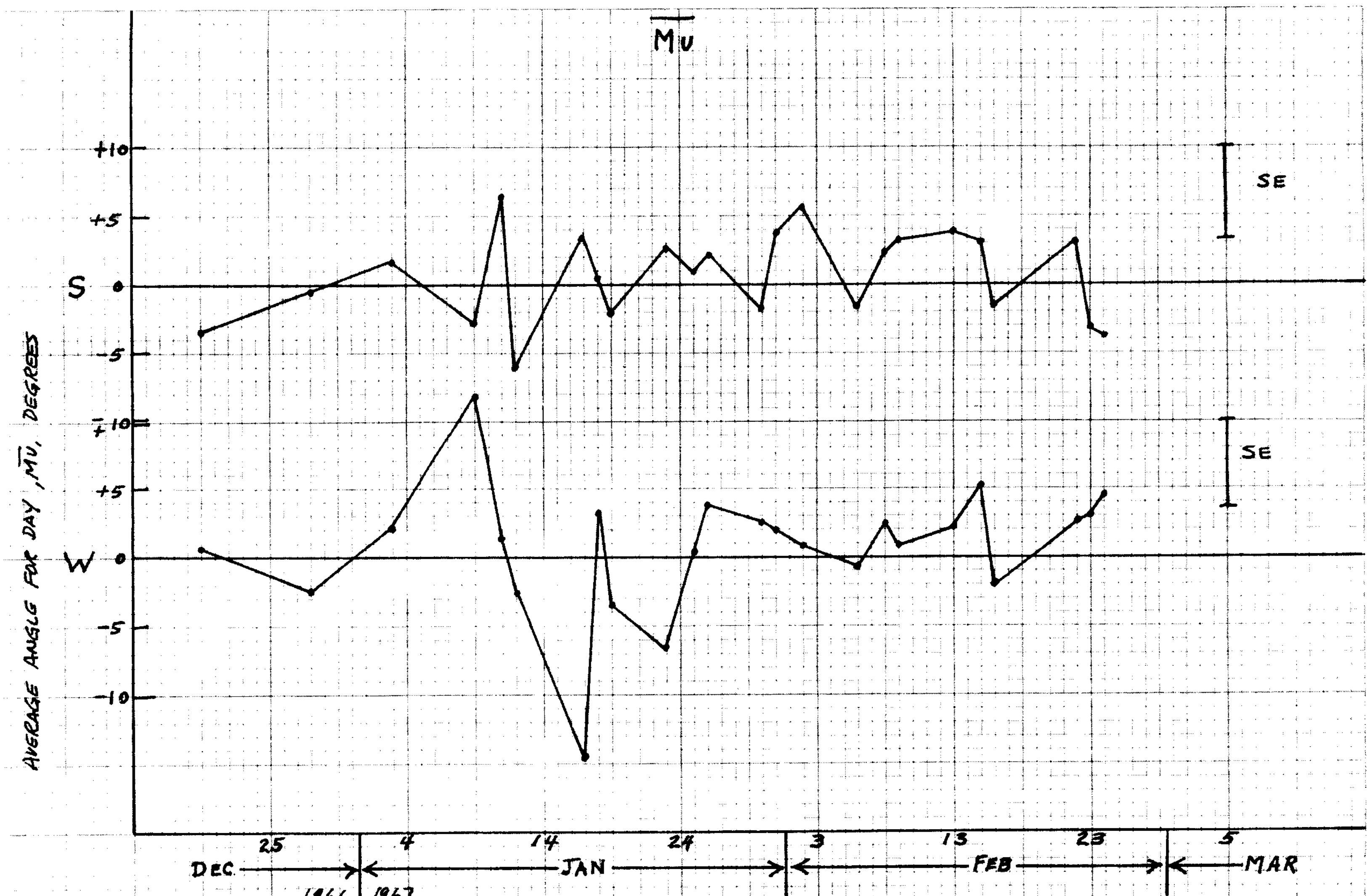


FIGURE 3.3-3 AVERAGE ANGLE FOR EACH DAY FOR SHIELDED CONDITION ($\overline{MU}$) FOR S & W INITIALLY DIRECTED WORMS WITH AVG. S.E.

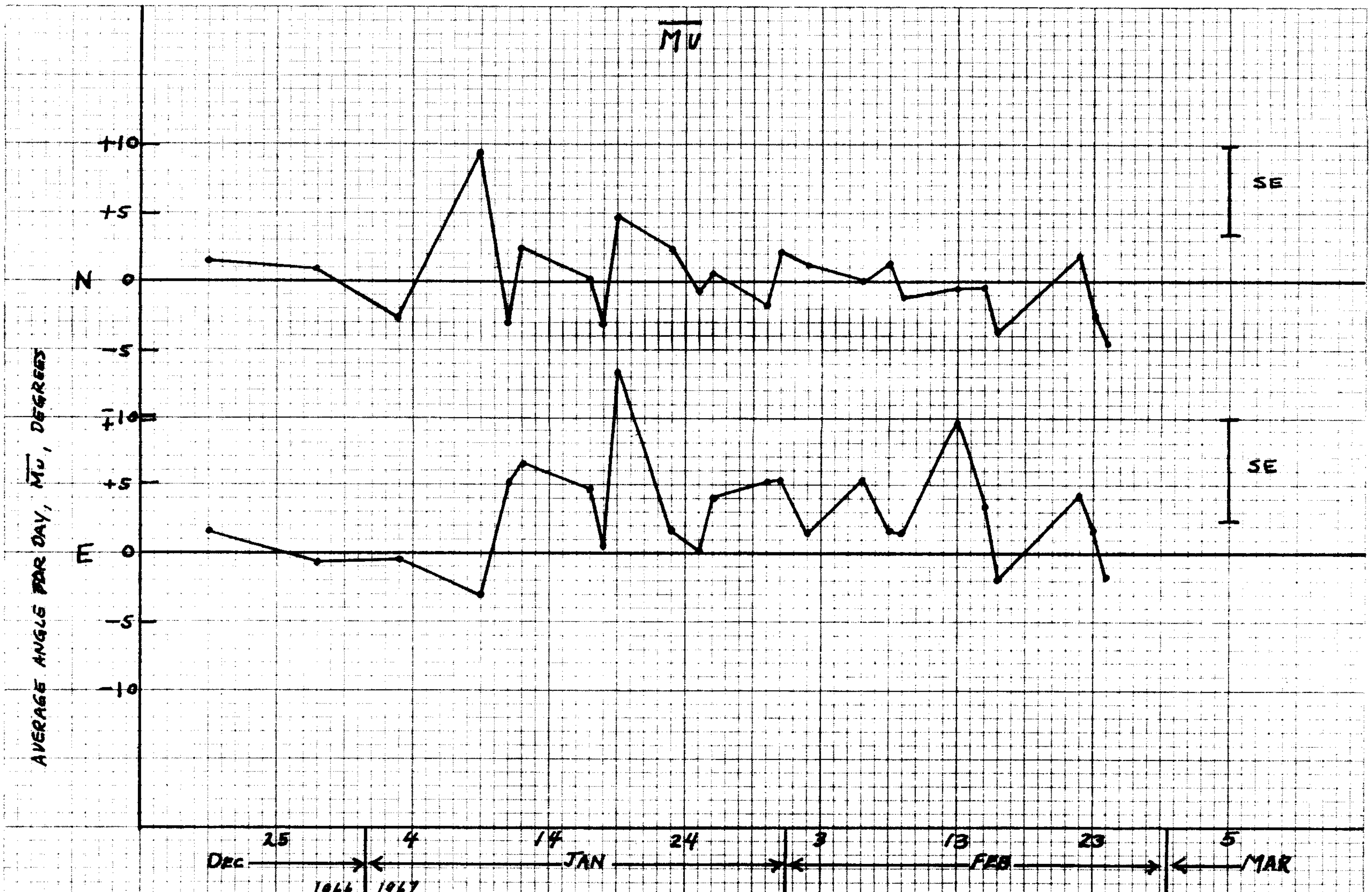


FIGURE 3.3-4 AVERAGE ANGLE FOR EACH DAY FOR SHIELDED CONDITION ($\overline{MU}$) FOR N & E INITIALLY DIRECTED WORMS WITH AVG S.E.

By forming this difference angle each worm thus serves as its own control, and any angular bias due to skewed light-beam angle or turning due to the phase of the moon is directly removed. Any after effect should also be removed by the random ordering and averaging over 18 worms. Thus the only stimulus left on the worm to influence its path is the difference in magnetic field between the shielded field and the normal field. However, one possible remaining difference was considered: This is the difference between the dishes used. Each worm makes one run on a clean dish. These dishes are identical molded plastic petri dishes. For each run a different cleaned dish is used. The size, shape, smoothness and cleanliness of each dish is, as far as could be controlled, identical. There is no expected variation due to the use of different dishes.

Since each worm acted essentially as its own control, and the difference angle (Al-Mu) was used, any natural right (or left) turning of a worm (independent of the experimental condition) was also effectively cancelled.

If there is no effect on the worm due to the magnetic field, the (Al-Mu) difference in any direction should have an expected value of zero. If we denote $(Al\text{-}Mu)_{ijn} = X_{ijn}$, then the average difference angle for any one day in one direction is X_{ij} with a Standard Error, S.E. (X_{ij}) and for the 24 experiments averaged is X_i and S.E. (X_i) for one direction. The variance of the mean is $V(X_i) = S.E.^2\ (X_i)$. This notation may be used interchangeably.

At this point it would be well to state in terms of our notation the hypothesis which we are testing.

$$H_o : X_i = 0 \quad (1)$$

$$H_i : X_i \neq 0 \quad (2)$$

Statement (1) says there is no difference in the average angle in any one direction obtained in the shielded (low magnetic field) and unshielded (normal magnetic field) conditions everything else remaining constant. The second (2) says: There is a difference, but we cannot specify whether it is a clockwise or counterclockwise turning.

In summary then the conditions are as follows:

Observations = 18 (the number of worms used per day)

Orientation = 4 directions: N, E, S, W

Replication = 24 (twenty-four days between December 20 and February 24)

Each observation consisted of the difference in the angles obtained from a pair of observations.

The linear model of the components of the criterion measure is

$$X_{ijn} = \alpha_i + \beta_j + \alpha\beta_{ij} + e_{ijn}$$

orientation $- \alpha_i \quad i = 1,2,3,4$

replication $- \beta_j \quad j = 1,2,\text{-----}24$

observation $- n \quad n = 1,2,\text{-----}18$

interaction - $\alpha\beta_{ij}$ (between orientation and replication)

error of measurement and variation of worms - e_{ijn}

Table 2 shows a summary of the statistics calculated

Sum of Squares	Variable	Mean Square Estimate of	Degrees of Freedom (df)
SS_A	α_i	$\sigma_\epsilon^2 + 18\,\sigma_{\alpha\beta}^2 + 432\,\sigma_\alpha^2$	3
SS_B	β_j	$\sigma_\epsilon^2 + 72\,\sigma_\beta^2$	23
SS_{AB}	$\alpha\beta_{ij}$	$\sigma_\epsilon^2 + 18\,\sigma_{\alpha\beta}^2$	69
$SS_{\omega cell}$	e_{ijn}	σ_ϵ^2	1632
SS_{tot}			1727

Table 2. Statistics Calculated in Analysis of Variance

Table 3 summarizes the calculations to be performed in the analysis of variance.

Variable	Description of Variable	Mean Squares to be Estimated	Degrees of Freedom	F-Ratio
α_i	orientation	$\sigma_\epsilon^2+18\sigma_{\alpha\beta}^2+432\ \sigma_\alpha^2 = A$	3	$F_1 = A/C$
β_j	replication (experiment day)	$\sigma_\epsilon^2 + 72\ \sigma_\beta^2 = B$	23	$F_2 = B/D$
$\alpha\beta_{ij}$	Orientation and replication interaction	$\sigma_\epsilon^2 + 18\ \sigma_{\alpha\beta}^2 = C$	69	$F_3 = C/D$
e_{ijn}	worm variation and experiment error	$\sigma_\epsilon^2 = D$	1632	
	Total		1727	

Table 3. F-Ratios Calculated and Degrees of Freedom Used in Analysis of Variance

If there is an effect due to orientation, replication or interaction, the appropriate F value for the proper degrees of freedom will be significantly greater than one. This is then an overall test of the total number of worm path differences over the $2\frac{1}{2}$-month period.

If F_1 is significant, then there must be an effect due to the orientation. If F_2 is significantly greater than one, there is an effect due to the day on which the experiment was performed (or alternately, since two operators ran worms, an effect due to the operator). Similarly if F_3 is significant, there is an effect which depends on the orientation and the day (or operator) acting together but is not necessarily a main effect due to either separately.

The analysis was performed by forming the difference angle for each of the 432 North directed pairs, 432 East directed pairs, 432 South directed pairs and 432 West directed pairs. These 1728 numbers along with their locations comprised the set of criterion measures for the overall experiment analysis. Following the Cornfield and Tukey procedure the crude sums of squares, sums of squares, mean square and F values were computed. These are summarized in Table 4.

A Wang Loci IIa programmable digital desk top computer was available at times in another department and was used to expedite the calculations required. The program capacity was not sufficient to program a complete analysis of variance (at least not readily). Therefore partial programs were written to obtain sums, sums of squares, standard errors, weighted means, variances and t values for various parts of the analysis.* This procedure greatly decreased the computation time and reduced the chance of errors. A half day's time on the computer was approximately equivalent to two or three days with a desk calculator. Table 4 gives the values of the statistics calculated and the probabilities of their occurrence by chance for (Al-Mu) criterion angles for 3456 worm paths and 1728 difference angles.

*Copies of programs and program cards are shown in Appendix 1.

Source of Effect	Sum of Squares SS	Degree Freedom df	Mean Square MS	F	Prob. %
Orientation	1799.832	3	599.944	2.44	7.4
Repl. (exp.)	7571.832	23	329.210	1.03	> 40
Repl. x exp.	16939.168	69	245.495	0.77	--
Variation of worms error, etc.	521.332	1632	319.444		
Total	547,642.832	1727	317.106		

Table 4. Analysis of Variance for the Criterion Measure (Al-Mu) for 1728 Differences in 3456 Worm Paths

The results of this analysis show that there is:

a. An effect due to orientation which is significant at the 10% level

b. No effect due to the replication (experiment run)

c. No interaction between orientation and experiment

The fact that there is no effect due to replication indicates that by using the difference angle (Al-Mu) we have successfully removed any general turning which was due to the time of month (moon effect or other time-dependent effect) and any effect due to the different techniques of the two experimenters.

Fig. 3.3-5 shows the averaged (Al-Mu) differences for each of the 24 experiments in each of the 4 directions and the weighted mean and its standard duration.

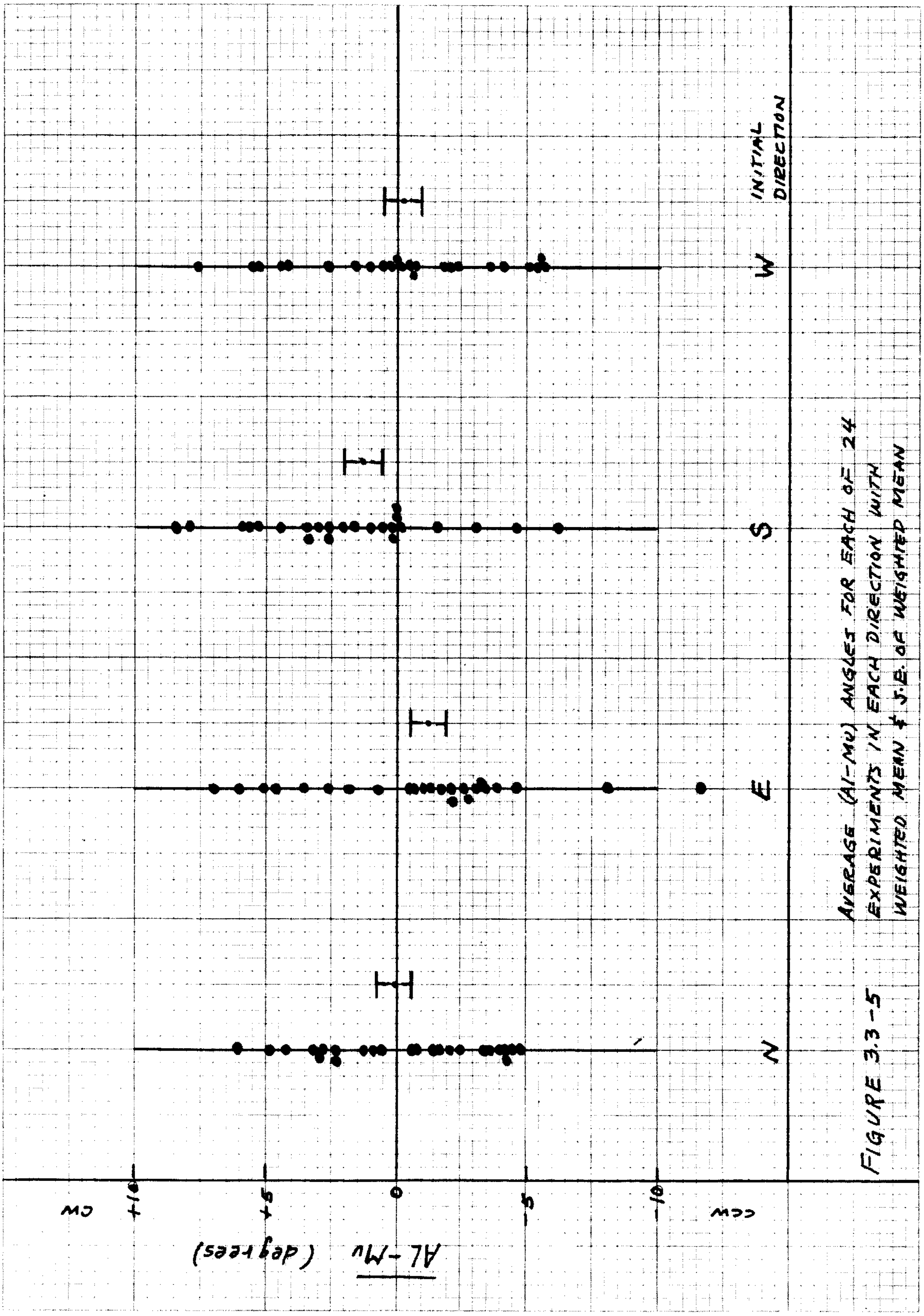


FIGURE 3.3-5 AVERAGE (AI-MU) ANGLES FOR EACH OF 24 EXPERIMENTS IN EACH DIRECTION WITH WEIGHTED MEAN ± S.E. OF WEIGHTED MEAN

It now remains to determine what the main effect is which is reflected in the significant F_1 (Table 5) and significance in each of the 4 directions. This is done with the t test. A summary of the mean turning angle difference, the standard error of the mean, the t value and the probability of obtaining this value by chance are summarized in Table 5.

Initial Direction of Worm	Mean (Al-Mu) Degrees	S.E. Mean	t	Prob. in % 1-Tailed for df - 431
North	- 0.273	0.860	0.32	37.5
East	- 0.940	0.883	1.07	14.2
South	+ 1.796	0.773	2.32	1.0
West	- 0.162	0.865	0.19	42.5
Avg. 4 directions	+ 0.105	0.428	-	-
Expected Angles	0	--	-	-

(+) indicates a clockwise turning, (-) counterclockwise as viewed from above

Table 5. Mean Values of Main Effects, (Al-Mu) for 4 Directions and Probabilities for 432 Differences or 864 Worm Paths

The t value is here defined by

$$t = \frac{X_i - X_e}{S.E.(X_i)}$$

The test is made against the expected value of the angle difference which is zero.

Where:

X_i is the average difference angle for 24 experiments with 18 worms each for each of the 4 directions

X_e is the expected value of this difference angle which under the null hypothesis is zero for all of the 4 directions

$S.E.(X_i)$ is the standard error of the mean computed for each of the 4 directions separately

A graph of the 24 averages for each direction with weighted mean and S.E. of weighted mean is presented in Figs. 3.3-6 and 3.3-7 plotted against day of experiment. Fig. 3.3-5 shows the same data (Al-Mu) for each of the 4 directions against direction with weighted mean and S.E. of weighted mean.

An analysis of the data from the standpoint of the two observers (P and I) revealed that the standard error of the one observer, P, was consistently higher than that of the other operator, I, by approximately a factor of 1.36. Out of the 24 experiments performed, P performed 14 and I performed 10. Since these were independent experiments it was felt that the mean angles could produce a better and more meaningful overall angle if the means were weighted over the 24 experiments. The best way to do this is to weight each mean by the reciprocal of its own variance. This yields the minimum variance for the mean (12).

X_{wi} weighted mean for one direction i = N,E,S, or W over 24 experiments, 18 worms each

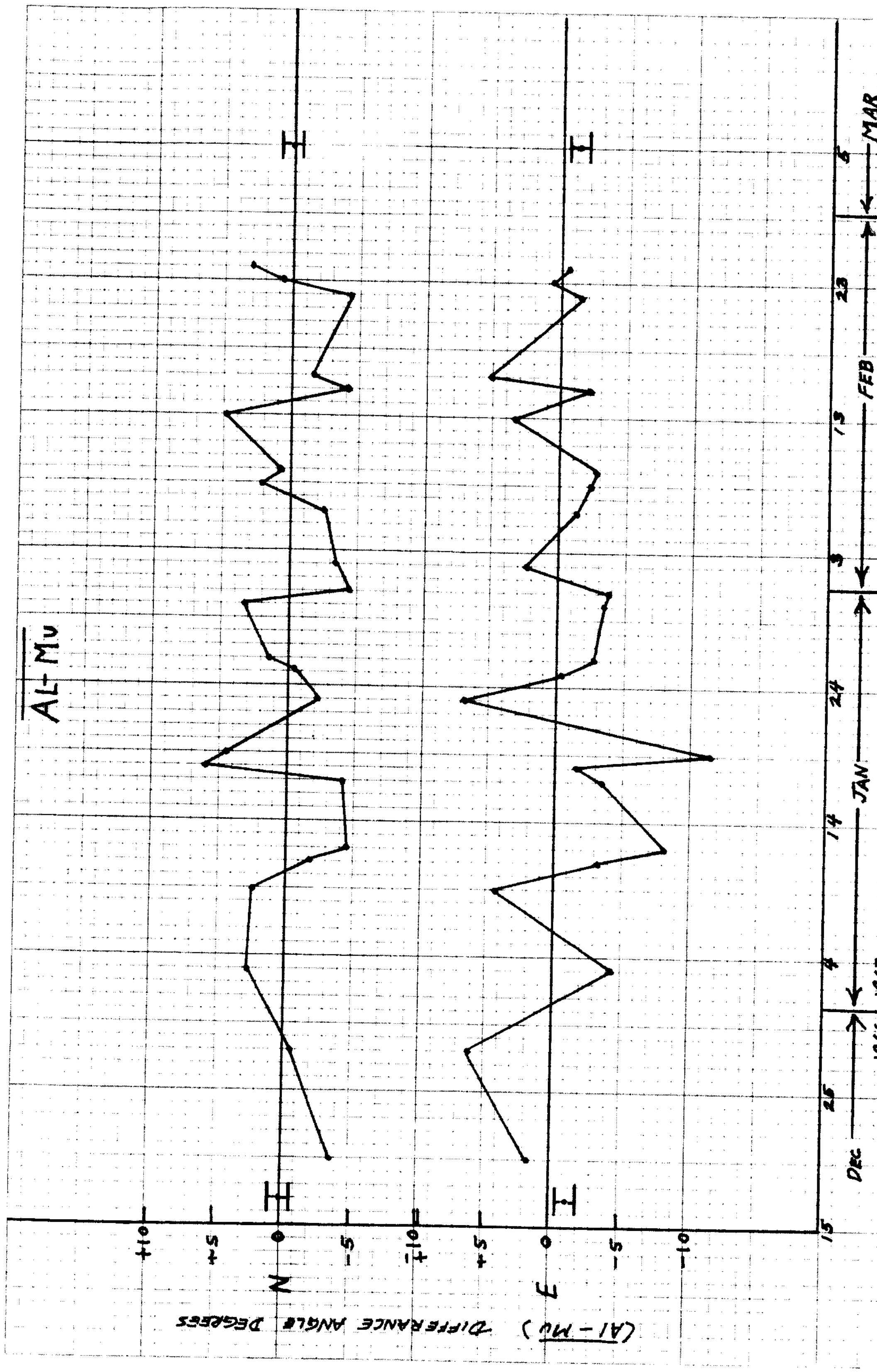


FIGURE 3.3-6 AVERAGE ANGLE ($\overline{AI-MU}$) FOR EACH DIRECTION VS. DAY OF EXPERIMENT WITH WEIGHTED MEAN AND S.E. OF WEIGHTED MEAN.

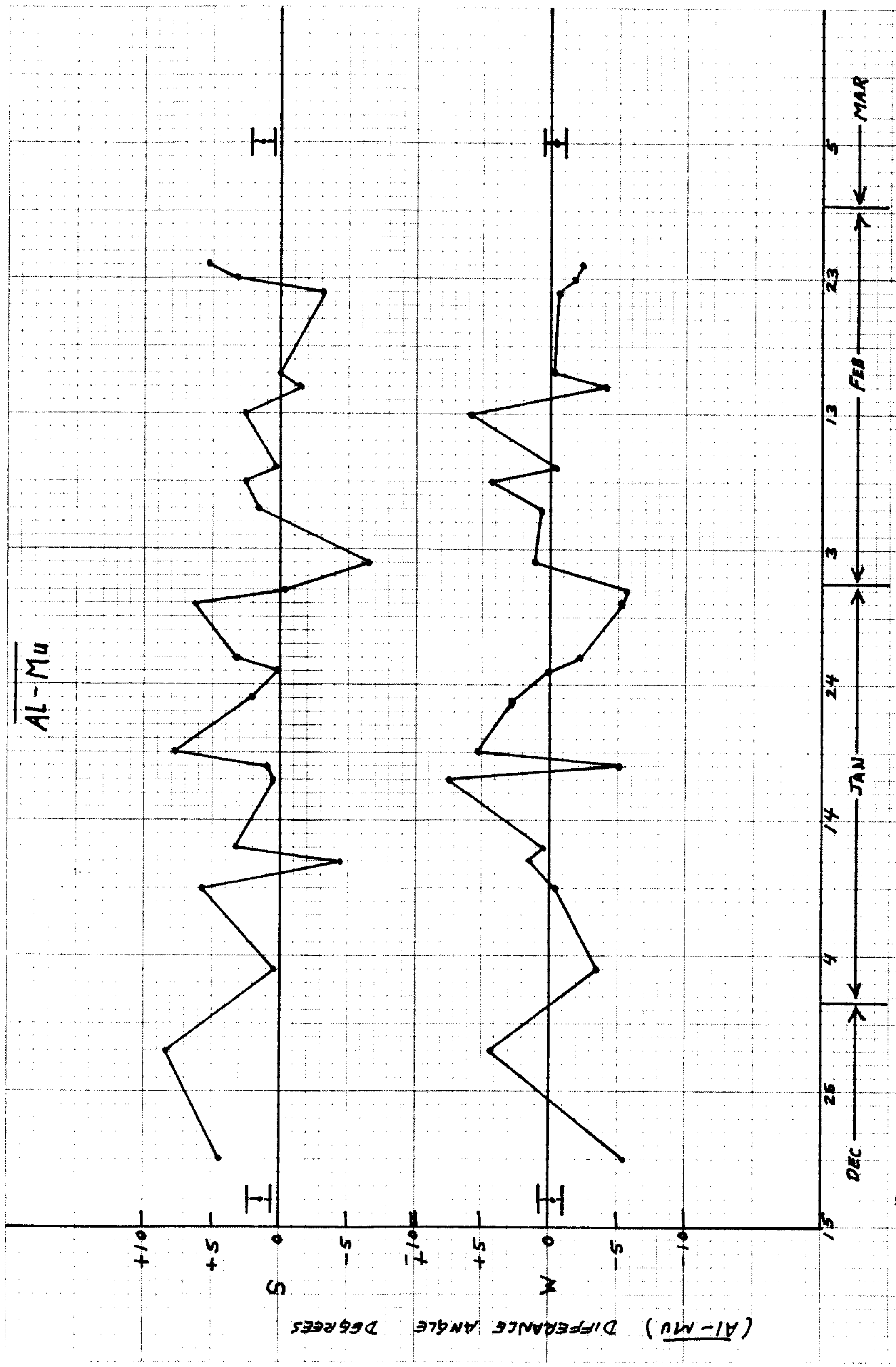


FIGURE 3.3-7 AVERAGE ANGLE ($\overline{AL-MU}$) FOR EACH DIRECTION VS. DAY OF EXPERIMENT WITH WEIGHTED MEAN AND S.E. OF WEIGHTED MEAN

$V(X_{wi})$ = variance = S.E.^{2} (X_{wi}) of weighted mean

$$X_{wi} = \sum^{24} \frac{X_{ij}}{V_{ij}} \Big/ \sum^{24} \frac{1}{V_{ij}}$$

$$1/V(X_{wi}) = \sum^{24} \frac{1}{V_{ij}}$$

Thus each day's mean difference angle was weighted by the reciprocal of its variance and divided by the total weight, and the variance of this weighted mean is the reciprocal of the total weight. Table 6 shows the results of these calculations

Initial Direction of Worm	Weighted Mean (Al-Mu) Degrees	S.E. of Mean	t	Probability in % 1-tailed df = 431
N	+ 0.037	0.763	0.05	48.0
E	- 1.178	0.730	1.61	5.4
S	+ 1.369	0.725	1.89	2.9
W	- 0.282	0.745	0.38	35.2
Weighted mean 4 directions	- 0.007	0.137	--	--
Expected Angles	0	--	--	--

Table 6. Weighted Mean Values of Main Effect and Probabilities (Al-Mu) for 432 Differences in each of 4 Directions

Here we see that the North mean angle is different in sign from that in the previous table, (Table 5), but these two values have no significance, whatever their signs.

The East direction is increased in significance while the South is decreased. Clearly in these two directions there was a turning which on the basis of the hypothesis and the design of the experiment can be interpreted in several ways; two of which are:

1. The worms attempted to align themselves away from a South-East line and direction when initially directed towards S or E.

2. The worms attempted to align themselves towards a NE-SW line without regard to direction but only when initially directed toward the S or E.

Other interpretations may be made, but we are not at the present justified in making any other than the most simple.

An interesting comparison can be made with the results obtained by Brown (4). The results obtained here for the S and E directions are the same as those obtained by Brown in direction of turning and approximate magnitude. The results obtained in the N and W directions do not directly contradict Brown, but neither do they confirm Brown's results. In the N and W while the direction of turning is the same as Brown's, clearly the magnitude (and thus significance of a turning angle) is not significant, and both magnitude and direction could easily have arisen by chance. Since, however, several aspects of the experiments done here were different from Brown's experimental procedure a direct comparison cannot be made.

A similar analysis of variance was applied to the 1728 worm paths in the shielded and unshielded condition separately. In this case a statistical procedure similar to that used in the (Al-Mu) difference angle was used but the criterion measure applied was the Al angle minus the average Al angle averaged over the 4 direction ($Al-Al_{avg}$) and for the shielded case the same, but the using the Mu angle average ($Mu-Mu_{avg}$). This is similar to the criterion measure used by Brown. These difference angles were averaged over 18 worms and analyzed over 24 experiments for the F test. These data are shown in Table 7.

Source of Effect ($Al-Al_{avg}$)	Sum of Square SS	Degrees Freedom DF	Mean Square MF	F	Prob. %
Orientation	1,925.753	3	641.918	4.44	< 1.0
Replication (exp)	652.896	23	28.387	< 1	-
Orientation and Replication	9,979.749	69	144.634	< 1	-
Worm variation and experiment error	338,120.672	1632	207.182		
Totals	350,679.070	1727	203.057		

Table 7. Analysis of variance for 1728 worm paths of the criterion measure ($Al-Al_{avg}$). Al_{avg} angle equals the average for 4 directions.

The difference angle ($Al-Al_{avg}$) and ($Mu-Mu_{avg}$) was formed for each observation by subtracting from each angle observation under no shield (Al) or shield (Mu) condition the average angle of the $18 \times 4 = 72$ angles recorded for that day. The average of these 72 angles thus effectively formed the control angle Al_{avg} or

Mu_{avg}. From this control angle the individual differences were formed and averaged to yield the day, or experiment average for each of the four directions. The results of the $Al\text{-}Al_{avg}$ analysis (no shield, ambient field) are shown in Table 8.

Initial Direction of Worm	$(Al_i\text{-}Al_{avg})$ Degrees	S. E.	t	Prob. % 1 tailed df = 431
N	- 1.261	0.691	1.82	3.4
E	+ 0.932	0.685	1.36	8.7
S	+ 1.464	0.604	2.42	0.6 0.8
W	- 0.202	0.657	0.31	37.8 37.8
Avg. 4 dir.	.233	0.343	--	--
Expected Angles	0	--	--	--

Table 8. The mean values of the main effects for the average deviations from the average of the four directions for 18 worms averaged over 432 worm paths in earth's ambient magnetic field

+clockwise, - counterclockwise

The analysis of variance table for the $(Mu-Mu_{avg})$ is given in Table 9 and the main effects and probabilities in Table 10.

Source of Effect $(Mu-Mu_{avg.})$	Sum of Squares SS	Degrees of Freedom df	Mean Square MS	F	Prob. %
Orientation	2,224.357	3	741.452	2.85	< 5.0
Replication (exp)	224.009	23	9.740	< 1	-
Orientation and Replication	17,934.194	69	259.910	1.26	> 25.0
Worm variation and exp. error	335,568.448	1632	205.618		
Totals	355,951.008	1727	206.109		

Table 9. Analysis of variance for 1728 worm paths of the criterion measure $(Mu-Mu_{avg})$. The Mu average angle equals the average for 4 directions.

Initial Direction of Worm	(Mu_i-Mu_{avg}) Degrees	S.E. of Mean	t	Prob. % 1-tailed df = 431
N	- 0.846	0.716	1.18	11.9
E	+ 2.024	0.720	2.81	2.5
S	- 0.607	0.654	0.93	17.6
W	- 0.124	0.663	0.19	42.5
Avg. 4 directions	+ 0.112	0.336	--	--
Expected Angles	0	--	--	--

Table 10. The mean values of the main effects for the average deviation from the average of the four directions for 432 worm paths in shielded cylinder or null field condition and their probabilities.

+clockwise, - counterclockwise

Since these are essentially the same data as before but merely processed in a different way, the statement regarding the variances of the two observers still applies and thus weighted means were calculated for these difference angles also. They are shown in Table 11.

Initial Direction of Worms	Weighted Mean (Al-Al avg.) Degrees	S.E. Weighted Mean	t	Prob. % 1-tailed df = 431
N	- 1.289	0.608	2.12	1.7
E	+ 0.711	0.539	1.32	9.3
S	+ 1.785	0.602	2.96	0.2
W	- .054	0.626	0.09	46.4
Mean Weighted	0.308	0.148	--	--
Expected Angles	0	--	--	--

Table 11. The weighted mean values of the main effects for the average deviation from the average of the four directions (for 18 worms) weighted by the reciprocal of the variances for 432 worm paths in the earth's ambient magnetic field

+clockwise, - counterclockwise

The weighted means and variances were computed similarly for the $(Mu\text{-}Mu_{avg})$ and are shown in Table 12.

Initial Direction of Worms	Weighted Mean $(Mu\text{-}Mu_{avg})$ Degrees	S.E. of Weighted Mean	t	Prob. % 1-tailed df = 431
N	- 1.728	0.634	2.73	0.3
E	+ 1.291	0.615	2.10	1.8
S	- 0.319	0.513	0.62	26.8
W	+ 0.224	0.566	0.40	34.5
Mean Weighted Expected Angles	0	--	--	--

Table 12. The weighted mean values of the main effects for the average deviation from the average of the four directions (for 18 worms) weighted by the reciprocal of the variances for 432 paths in the shielded condition

+clockwise, - counterclockwise

Generally the weighting increases t values which are large and decreases those that are small, apparently bringing out the true effect more clearly.

The $(Al\text{-}Al_{avg})$ difference angles clearly show an effect in three directions: N, E, and S.

The $(Mu\text{-}Mu_{avg})$ difference angles clearly show an effect in two directions, N and E. The use of $(Al\text{-}Al_{avg})$ and $(Mu\text{-}Mu_{avg})$ as the criterion measure is discussed later in the report.

3.4 CONCLUSIONS AND DISCUSSION

3.4.1 DISCUSSION

As shown by Brown there is a cyclical turning due to the lunar effect. By forming the difference angle between the angles observed in the Al and Mu cylinders (no shield and shield) this response in the present experiment should be completely eliminated. Any skewing due to the apparatus should also have been completely eliminated. The expected angles under the null hypothesis should then be zero. In the analysis where the ($Al-Al_{avg}$) angle and the ($Mu-Mu_{avg}$) angle was used we would expect the lunar response to be eliminated only when there was a constant uniform lunar response for any direction of travel or a response with certain boundary conditions in both magnitude and direction for direction of travel (See Appendix 1). In these cases the angle-angle (avg. 4 directions) should effectively remove the lunar response.

The average response of the worm in the (Al-Mu) analysis with weighted mean is not symmetrical with respect to orientation. In both the south and east orientations a nearly significant effect of slightly over 1° is found. The turning is <u>away</u> from the south-west direction, (Fig. 3.4-1). This was not anticipated. In the west and north directions not even a slight trend is noted as shown by the large values of P for these directions. There are various possibilities as to why this pattern of response occurred. One possibility is that the shielding was not sufficently great and that

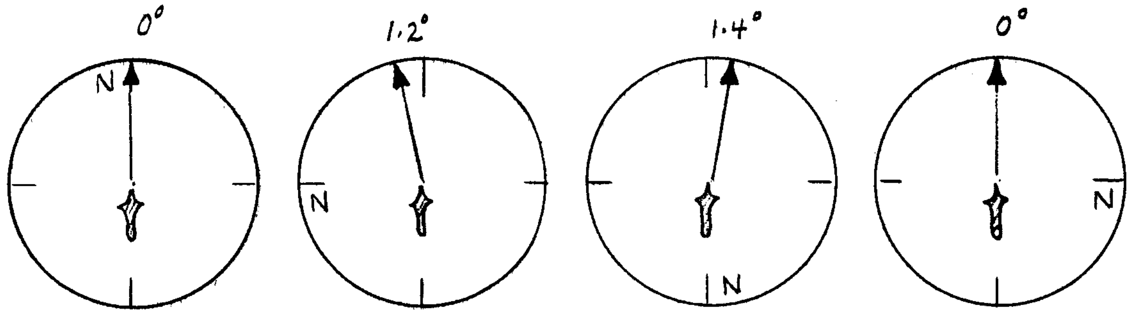


Fig. 3.4-1 - From Table 13 Mean Differences of Turning Angles (Al-MU) for 3456 Paths. N Indicates Magnetic North.

a small residual field (not necessarily in the same direction as the earth's field remained and was sufficiently large to produce a response. This would require that the worms be sensitive to fields of less than five millioersted and that their response is not linear with respect to magnitude. Essentially their response might be nearly the same for any field above a certain minimum.

The analysis of the ($Mu-Mu_{avg}$) (all data from shielded condition) angles shows a definite orientation effect. The analysis of variance P for the main effect is < 5% compared to 7.4% for (Al-Mu) and < 1% for ($Al-Al_{avg}$). This would indicate that there is still an orientation effect even under the shielded condition. It further indicates the orientation effect without the shield ($Al-Al_{avg}$) is considerably stronger. The fact that there is a fairly significant effect for the (Al-Mu) angles indicates that the shield was effective to some extent, at least partially removing the earth's magnetic field. The evidence of an orientation effect in the Mu shield was not expected.

A summary of the results from the three analyses is given in Table 13 with weighted mean angles in degrees and probabilities in per cent.

	No Shield - Shield $(Al\text{-}Mu)_W$	All No. Shield $(Al\text{-}Al_{avg})_W$	All Shield $(Mu\text{-}Mu_{avg})_W$
N	+ 0.04 (48%)	- 1.29 (1.7%)	- 1.73 (0.3%)
E	- 1.18 (5.4%)	+ 0.71 (9.3%)	+ 1.29 (1.8%)
S	+ 1.40 (2.9%)	+ 1.79 (0.2%)	- .32 (26.8%)
W	- 0.28 (35%)	- 0.05 (46%)	+ 0.22 (34.5%)
Expected Angles	0	0	0

Table 13. Summary of results of three methods of analysis and their probabilities

+ clockwise, - counterclockwise

These values can be interpreted as a turning away from a SE direction for $(Al\text{-}Mu)_W$, a turning toward a SW direction for $(Al\text{-}Al_{avg})$ and a turning away from a NE direction for $(Mu\text{-}Mu_{avg})$. This can be illustrated qualitatively in Fig. 3.4-2 where paths taken are shown dotted and the angle is exaggerated.

A word of caution must be interjected here, however. It will be noticed that in the analysis of variance of the $(Al\text{-}Al_{avg})$ and $(Mu\text{-}Mu_{avg})$ data the MS or mean square values of the replication are unnaturally small. The expected value of these mean squares is about the same size as the mean square for worm variation and experimental error. At present this discrepancy cannot be accounted for. The procedure and computation was checked for

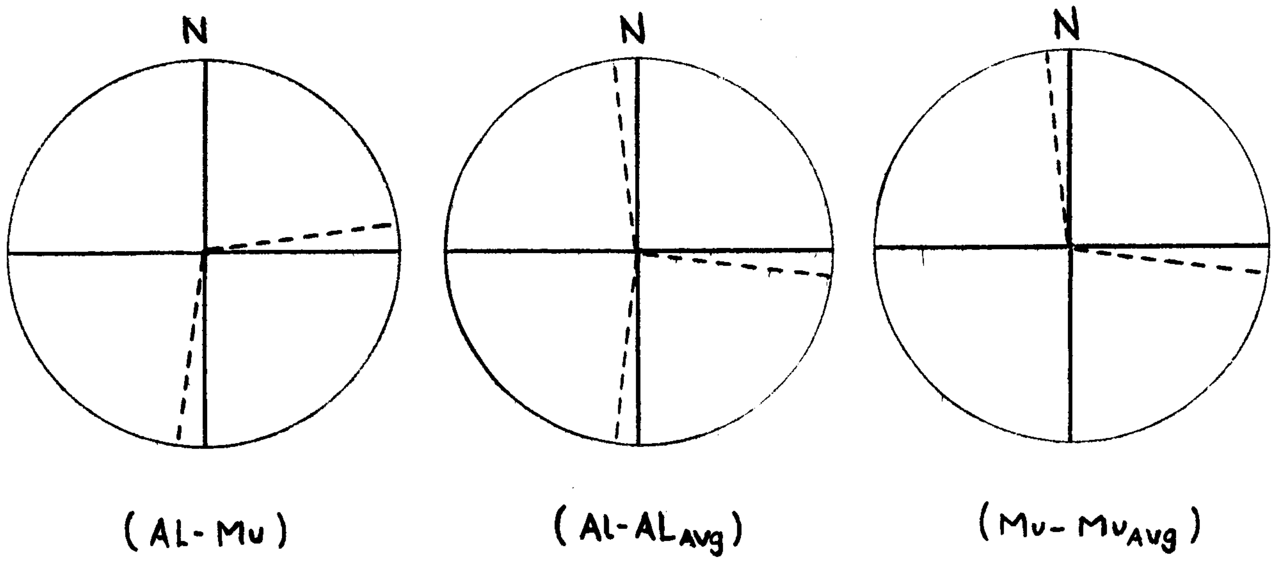


Fig. 3.4-2 - Qualitative Representation of Results for the Three Methods of Analysis

error, but nothing of significance was found. Because of this the values obtained in the $(Al-Al_{avg})$ and $(Mu-Mu_{avg})$ analysis should be treated with some suspicion whereas those from the (Al-Mu) analysis are considered completely valid because of their internal consistency.

3.4.2 CONCLUSIONS

The following conclusions may be drawn from this experiment. An overall effect on the navigation of planaria was found which can be directly attributed to the earth's magnetic field. The probability of this occurring by chance was less than 10% (7.4%). It consisted of a small clockwise turning of the worms when directed South and a small counterclockwise turning when they were directed east; no effect was noted when they were directed north or west. The effect detected was the difference in angle of travel when the worms were exposed to the earth's magnetic field and when they were shielded from it. No effect was noted which could be attributed to the replication (experiments extended over $2\frac{1}{2}$ months) or to an interaction effect between replication and orientation. No direct comparison can be made between these results and those of other investigators because the experiments performed were not sufficiently the same, although similar in some respects.

3.4.3 OTHER POSSIBLE EXPERIMENTS

While the experiment performed here undoubtedly produced a result, it required the average of a very large number of measurements and extensive and time-consuming analysis. This may be due to one or more of the following conditions: The response to a magnetic field is very small in all animals, the response is found in only a small percentage of animals, the response varies greatly between animals but is found in all to some extent, or the response is positive in some animals and negative in others and to varying degrees. It would be greatly advantageous to have a method of assay which would be more sensitive than the present method and would have a simple binary or two-choice output. Such a possibility may exist in an apparatus which gives the animals only two choices rather than essentially infinite number of possibilities. In the early stages of our work numerous methods of guiding the worm in its initial direction were tried. Guiding tubes, various geometrical shapes of runway, etc. were tried and discarded in favor of manual orientation by gently pushing the worm. It is felt that this is somewhat confusing or disturbing to the worm and that if left to its own devices, but guided by a channel of the proper sort, the variation in path angle could be greatly decreased and thus the precision of measurement increased with a consequent reduction in the number of paths required for a given confidence level. Further, the analysis could probably be carried out with greater ease and speed. It is felt that some kind of "Y" or "T" tube or channel can be constructed which will provide these advantages. If this can be developed, many changes in the magnetic field levels and directions could be rather quickly treated, as well as changes in other factors.

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4.0 MULTI-CHANNEL TELEMETRY

4.1 PHILOSOPHY OF DESIGN

Our fundamental philosophy has not changed and remains essentially as expressed in our previous annual report (4-1).

Our findings during this year of effort have strengthened our belief that the goals outlined in the referenced report can be reached. Substantial progress has been made.

4.2 MATERIALS

4.2.1 ENCAPSULANT DEVELOPMENT

We have continued leakage tests of Mixture 26. Figure 4.2-1 illustrates results. The immersion period of 9880 hours (at the present) represents 1.14 years. Much experience has been gained with mixture 26. A substantial number of implants were made in the Rhesus monkey (at no cost to this contract), and encapsulant failure was minimal. In all cases the temperature sensor involved was a thermistor located at the distal end of a long, slender umbilical which was thinly coated with Mixture 26.

We have also sent sample quantities of Mixture 26 to the following researchers:

Dr. Frank Craighead, Jr., President
Environmental Research Institute
Moose, Wyoming

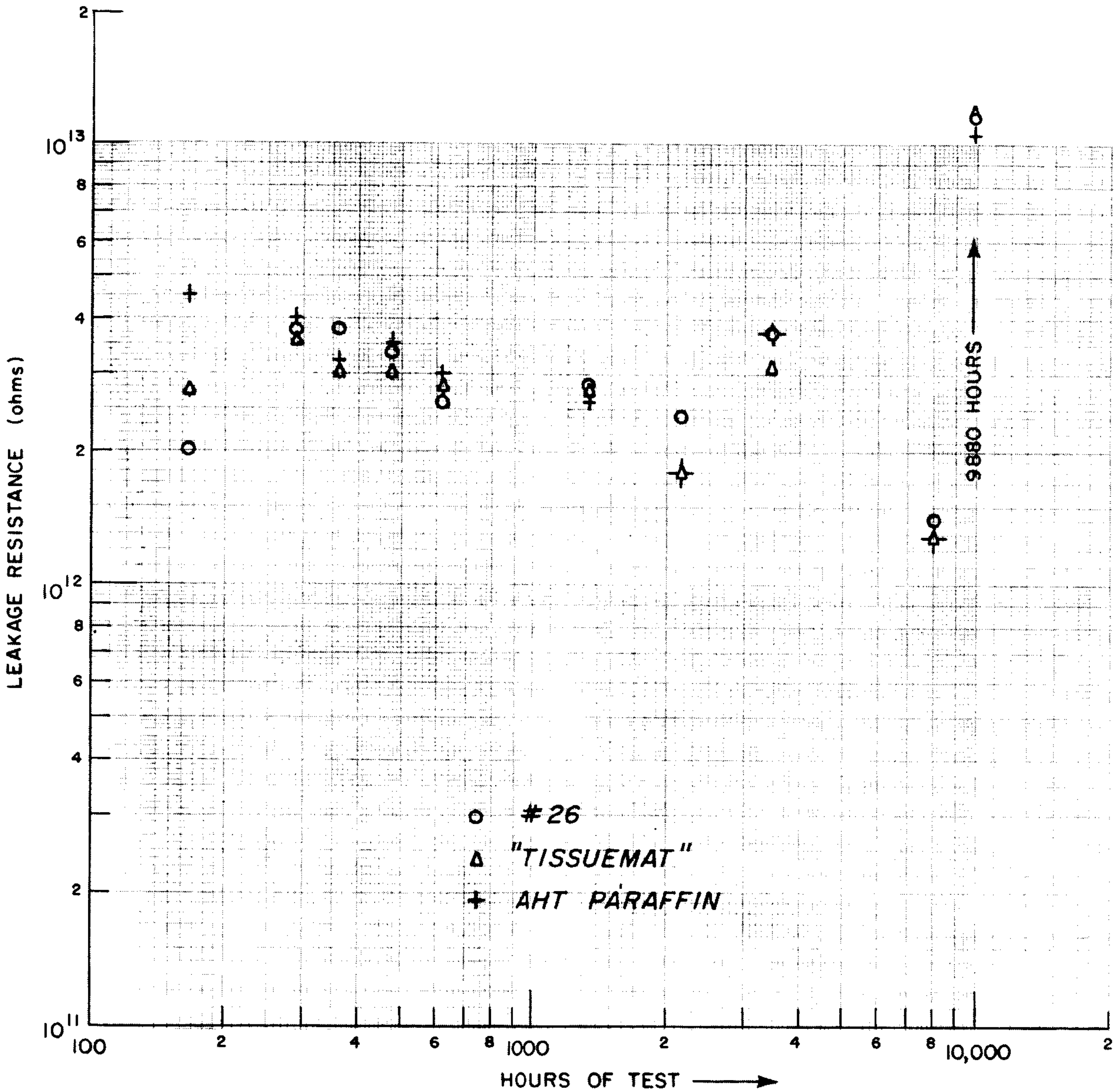


Fig. 4.2-1 - Leakage Test Data.

Dr. Wen H. Ko, Associate Professor
Case Institute of Technology
Cleveland, Ohio

Dr. Lyman Fourt, Assistant Director of Research
Harris Research Laboratories
Washington, D. C.

Dr. Clement S. C. Lear
Forsyth Dental Center
140 Fenway
Boston, Massachusetts 02115

Dr. Patrick J. Carr
National Institutes of Health
Bethesda, Maryland

Dr. Gerald Vurek
National Institutes of Health
Building 10, Room 5-D-18
Bethesda, Maryland

Dr. Peter Carmeci
Head, Engineering Support Division
Defense Atomic Support Agency
Armed Forces Radiobiology Research
Bethesda, Maryland 20014

Dr. Maitland Baldwin
National Institutes of Health
Bethesda, Maryland

To date, we have the following reports on performance:

a. Dr. Wen Ko: Excellent results have been obtained with Mixture 26. Ko wishes to obtain larger quantities throughout the year, (4-2).

b. Dr. Lyman Fourt: The material is in the class with polyethylene silicone with respect to its effect on clotting time of human citrated plasma (with "Tris" buffer pH7.3) at isotonic concentration. Clotting time was measured from time of addition of $CaCl_2$ solution, (4-3).

We will report other responses as we receive them.

4.2.2 ELECTRONIC COMPONENTS

Changes in component selection have been made since the referenced report (4-1) was written. In order to improve our space-volume economy we have shifted over to the use of resistors available from British Radio Electronic Industries, Ltd.

These units are modest in cost and considerably smaller in size than the Allen-Bradley units originally selected. Availability of higher value BREI units in the future will further improve our space economy. We remain, of course, sensitive to the availability of special, ultraminiature, thick-film resistors and to the continuous improvement in the MOS-FET transistor for future resistor applications.

Ceramic-chip capacitors similar to those supplied by Gulton and others have proven satisfactory. We do not foresee a rapid shift from these units.

Our experience with semiconductors, while not discouraging, has been frustrating. A number of devices have been obsoleted by the manufacturers—even in cases where assurances were had that "it couldn't happen". However, we see no insoluble problems (yet) arising as a result of such changes.

4.3 MECHANICAL DESIGN

Fig. 4.3-1 illustrates the basic chassis design for the two-channel temperature telemeter, Mark V/2(T,T). Fig. 4.3-2 provides dimensional details. This chassis is designed with a "wiring deck" on each side and a center hole to accept a mercury cell. The wiring deck permits incorporation of interconnecting wiring with ease and at the same time precludes the possibility of any wires protruding above the maximum height of the telemeter chassis.

The peripheral groove on the outside of the body annulus has been designed to hold exactly two turns of No. 24 wire—the oscillator tank coil and in this case, the radiating element. The groove depth is such that the coil wires do not extend beyond the maximum diameter of the telemeter chassis.

4.3.1 CHASSIS FABRICATION

Fig. 4.3-3 illustrates how the chassis of Fig. 4.3-1 has been machined for acceptance of components and wiring.

The technique used was simple (in retrospect) and saves the immense effort involved in a detailed dimensional scale drawing and subsequent precision machining. In our case, we first designed a mechanical layout as illustrated in Fig. 4.3-4. This layout includes all components and all wiring. A similar layout was made for a three-channel telemeter. Original layouts were all ten-times actual size and were made with reasonable accuracy. Fig. 4.3-5 shows the layout for a three-channel unit.

On completion, the X10 size layout was accurately photographed and then reduced to actual, or X1 size. Reduction was made on clear film so that components and all center marks could be clearly seen under the microscope. The X1 size transparency was then glued to the plastic chassis and holes and/or depressions for feed-through leads and components were machined by using a precision jeweler's drill, dental tools and a binocular microscope. One must remember that this effort is developmental. Once we are satisfied with a design and its performance, a number of schemes are available for reproducing plastic chasses by molding rather than machining.

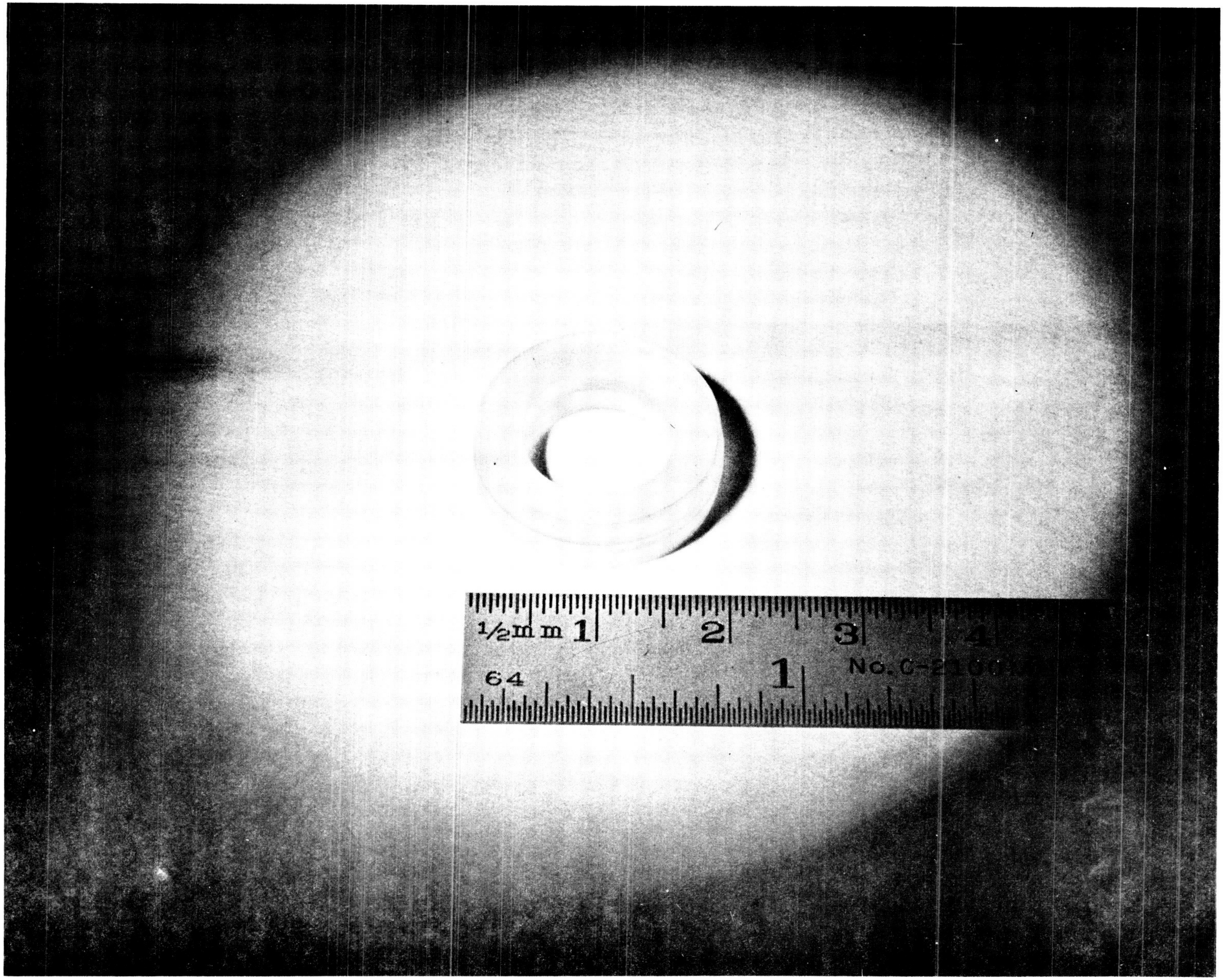


Fig. 4.3-1 - Mark V, 2-Channel Chassis

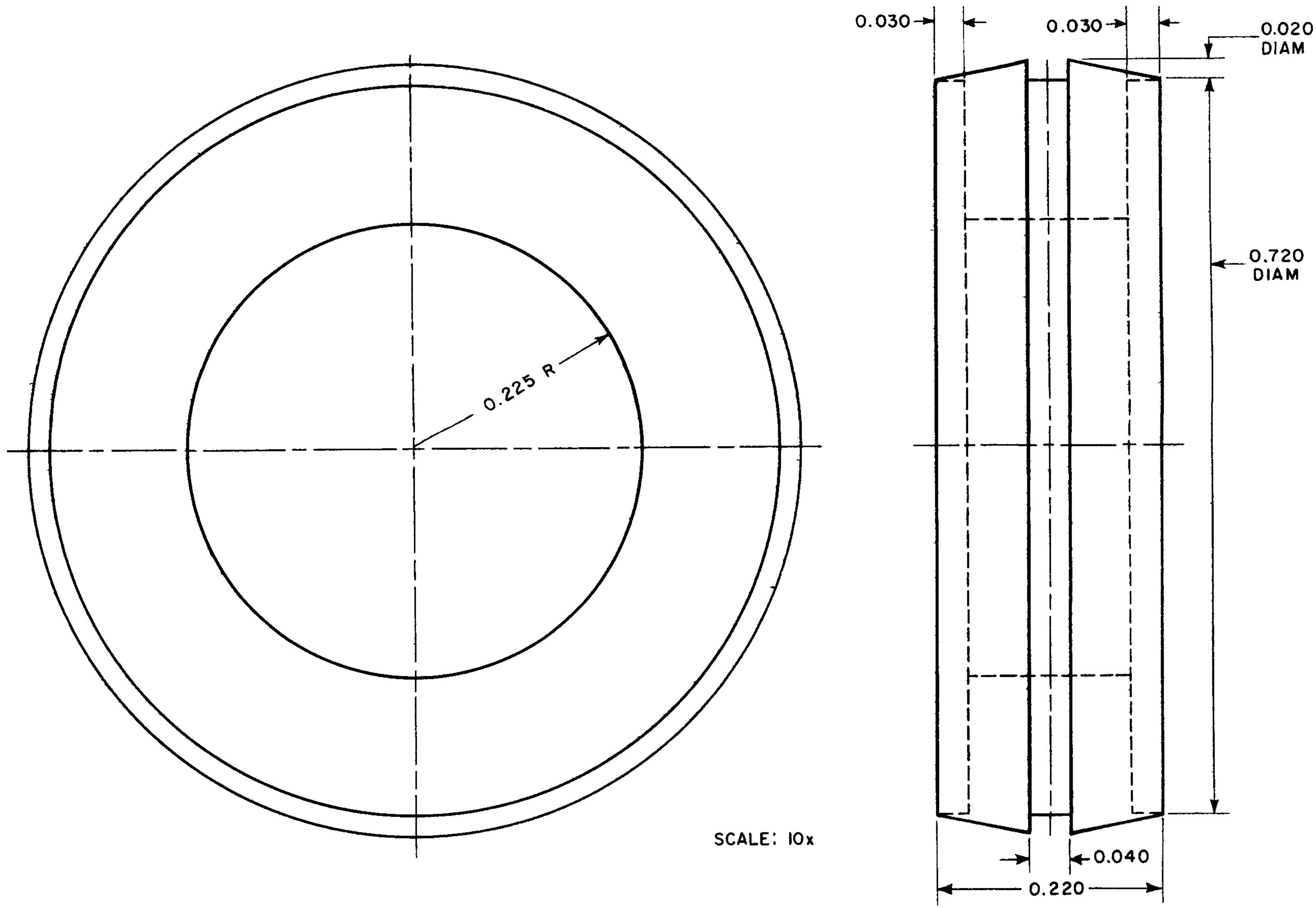


Fig. 4.3-2 - Mark V, 2-Channel Chassis

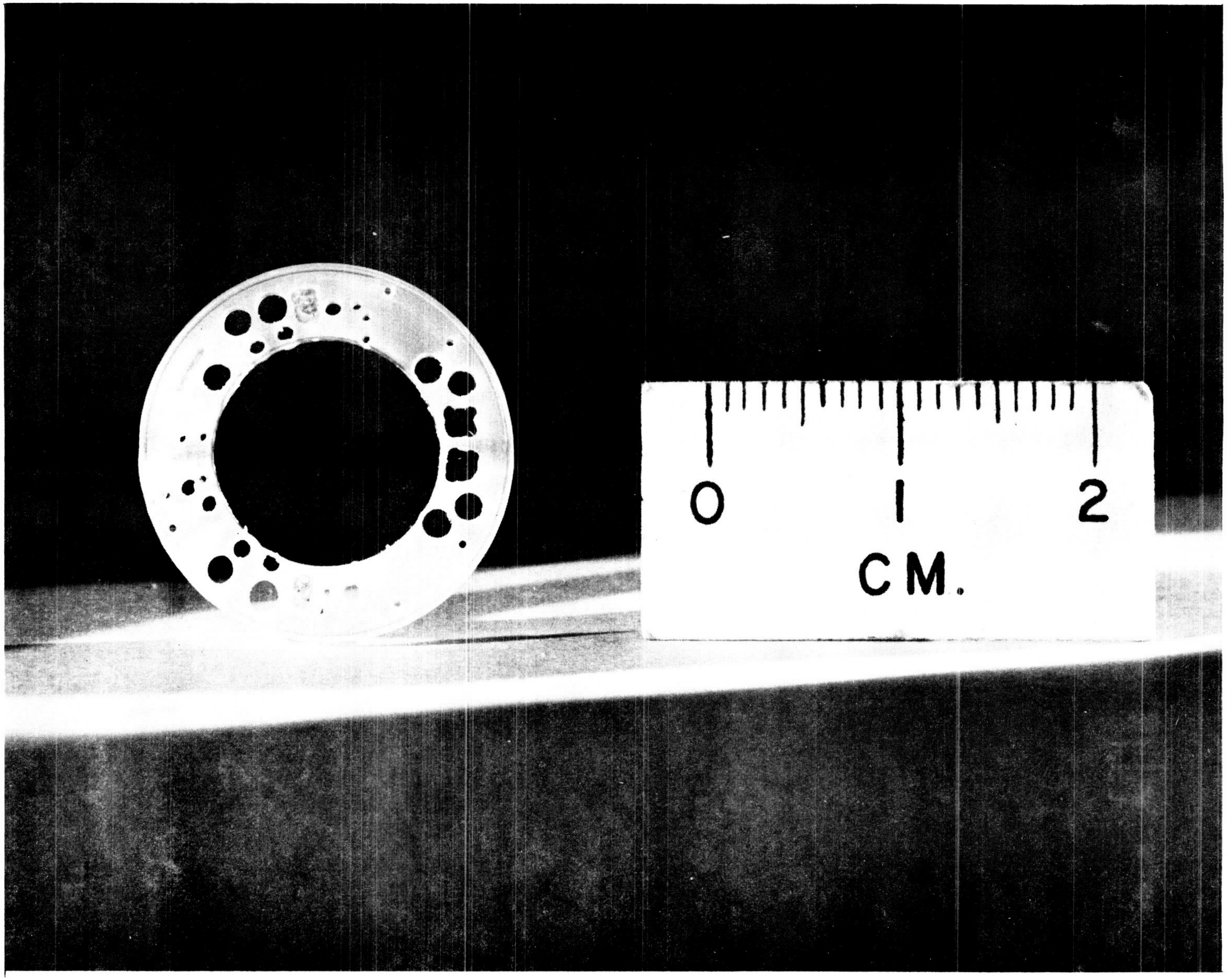


Fig. 4.3-3 - Mark V, Machined Chassis

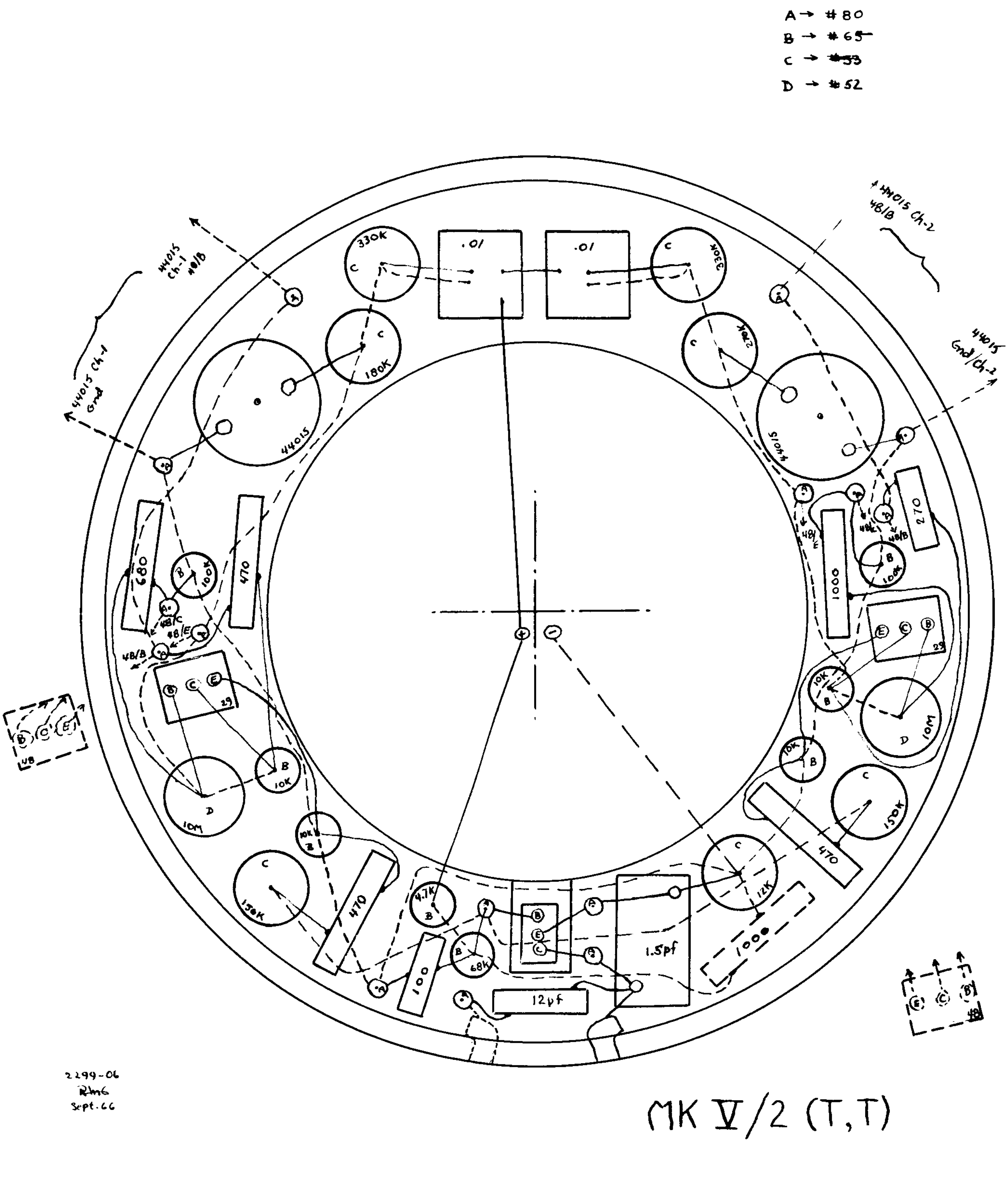


Fig. 4.3-4 - Mechanical Layout, 2 Channel Mark V (Size X10)

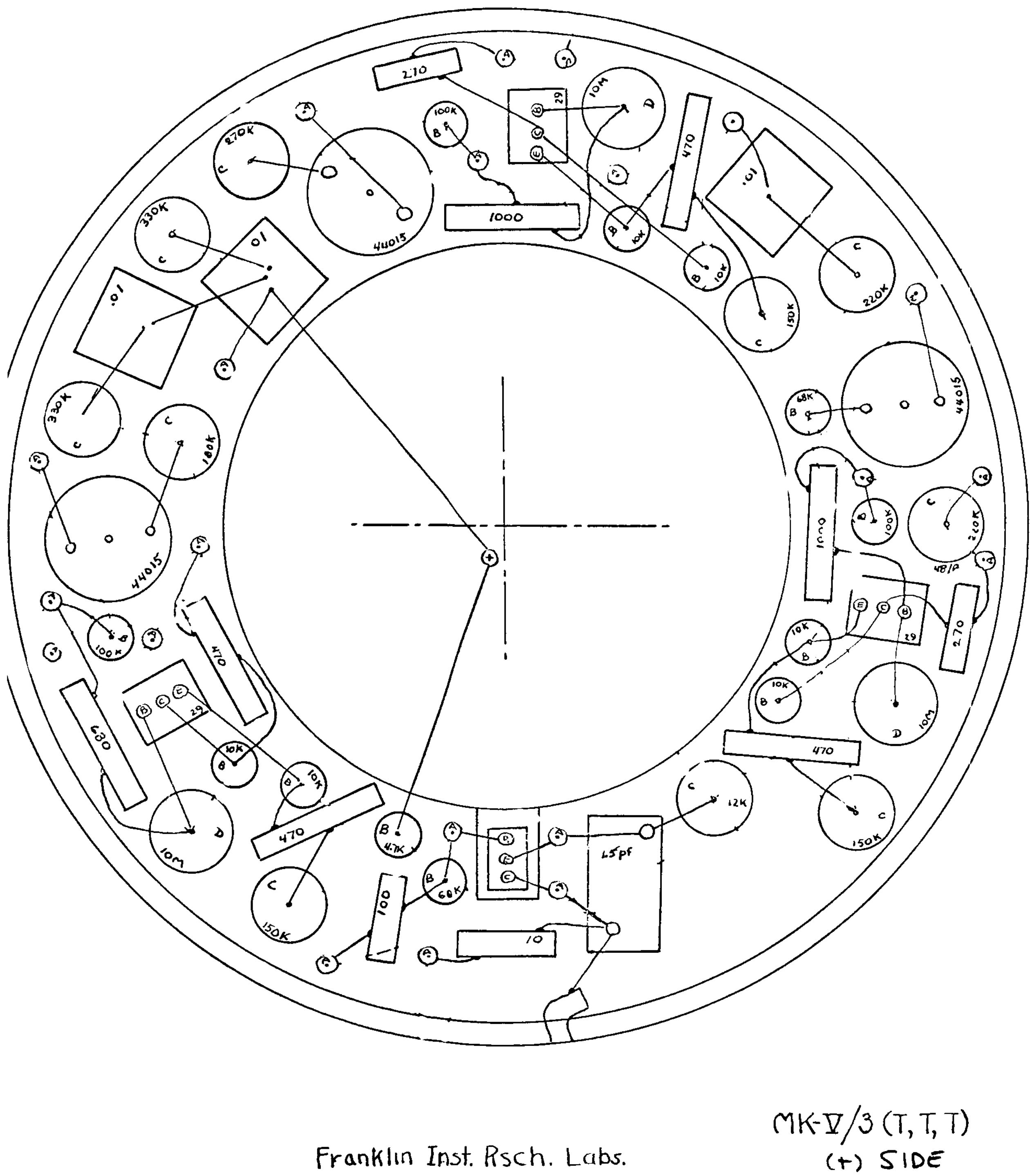


Fig. 4.3-5 - Mechanical Layout, 3 Channel Mark V (Size X10)

Figs. 4.3-6 and 4.3-7 illustrate X1 prints of a two-channel and three channel fm/fm layout. It is pertinent to observe that in the last sixty-day period these layouts have been obsoleted by the availability of certain improvements in component size. Fig. 4.3-8 shows the transparency template in place on a chassis just prior to machining.

The rapidly machined chassis is shown in Fig. 4.3-3.

4.3.2 WIRING

To save space and to simplify wiring, both sides of the chassis were used for circuit connections. Feed-through bus leads were made of 14.5 mil tinned wire, and interconnecting wire (hookup wire) was 3.5 mil tinned wire. All joints were soldered using a simple temperature-controlled, foot-switch-operated iron with a 10 mil tip. Gold-plated solder balls in the size range of 5 to 15 mils in diameter were used. All joints were fluxed with an activated rosin material. All soldered joints were cleaned with alcohol. All wiring and soldering was accomplished with the aid of a binocular dissecting microscope and was surprisingly easy to process.

Figs. 4.3-9 and 4.3-10 show each side of the wiring decks of the Mark V. Relatively simple wiring patterns will be noted as will be the cleanliness of the joints.

While the final connections from the circuits to the mercury cell were made by soldered joints, note that actual contact to the cell

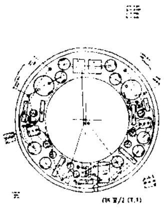


Fig. 4.3-6 - Mechanical Layout, 2 Channel Mark V (Size X1)

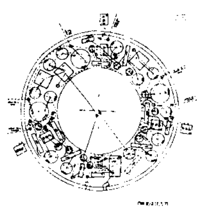


Fig. 4.3-7 - Mechanical Layout, 3 Channel Mark V (Size X1)

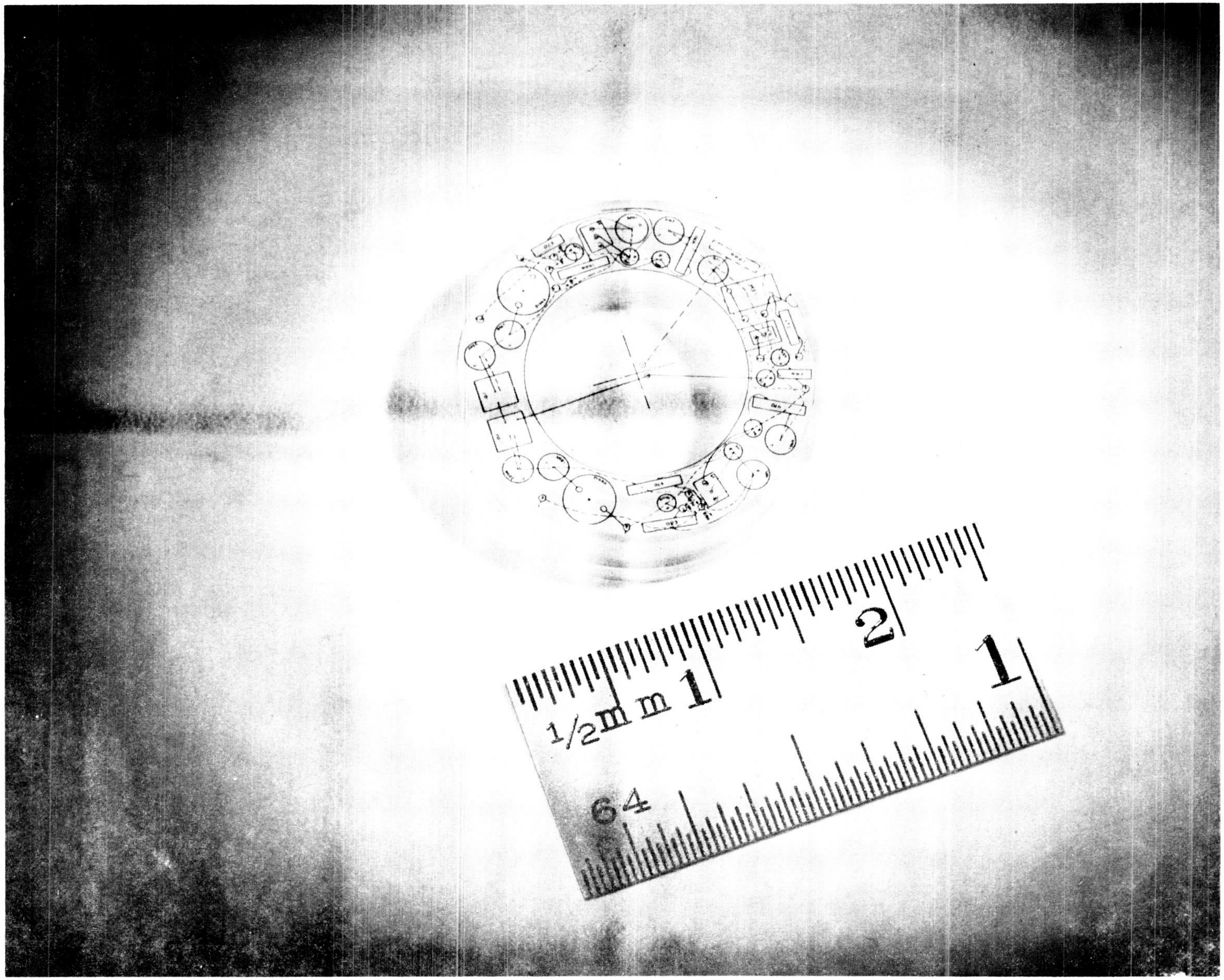


Fig. 4.3-8 - Template and Chassis, Mark V

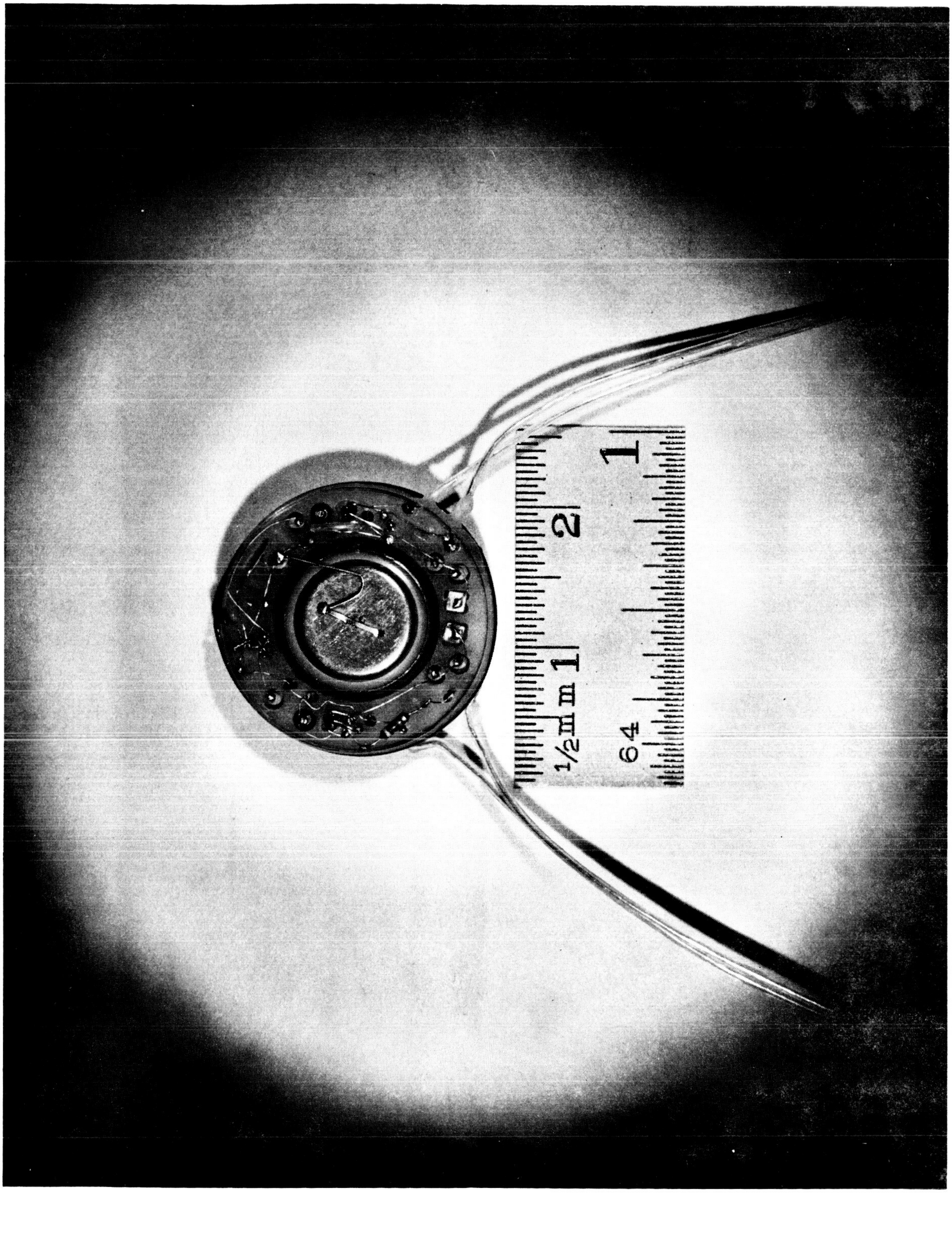


Fig. 4.3-9 - Wiring, Mark V, Negative Side

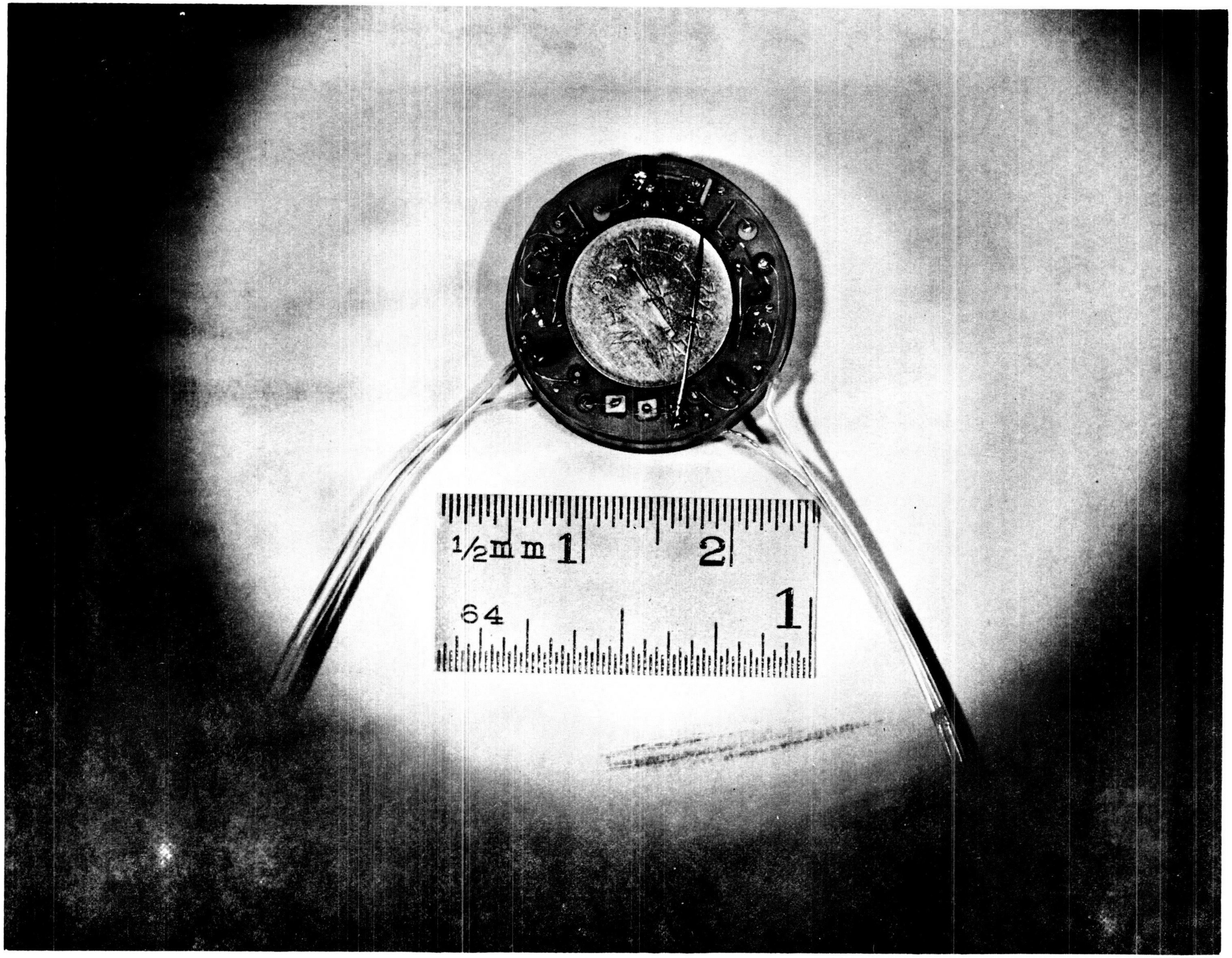


Fig. 4.3-10 - Wiring, Mark V, Positive Side

itself is via a series-welded nickel ribbon.

The antenna and tank coil consist of two turns of No. 24 Formvar wire wound in a precut chassis groove. This coil is actually made by precutting the wire to length, pre-tinning its ends and then winding.

Umbilical leads with distally located sensors are ruggedly attached to the telemeter by soldering to feed-through bus "bars" after insertion of the umbilical leads through tiny holes in the rim of the wiring decks. The individual umbilical leads are protected by polyethylene tubing which is compatible with encapsulant 26.

4.3.3 CHASSIS ENCAPSULATION

Let us refer briefly to Fig. 4.3-11 which shows diagramatically the plan for total encapsulation of the chassis.

The final unit approaches this design quite closely with only minor variations.

In the first place, we decided against the use of hard epoxy directly on circuit components because of their susceptibility to fracture by epoxy-shrink or cure. Ultimately, however, we plan to utilize a thin coating of resilient epoxy and then a final coating of hard epoxy. At present, we used a total wiring-deck full of clear Sylgard, a flexible and relatively rugged silicone compound. For present purposes this coating provides necessary component protection, permits component viewing (because of its transparency) and does not introduce severe stress to delicate components.

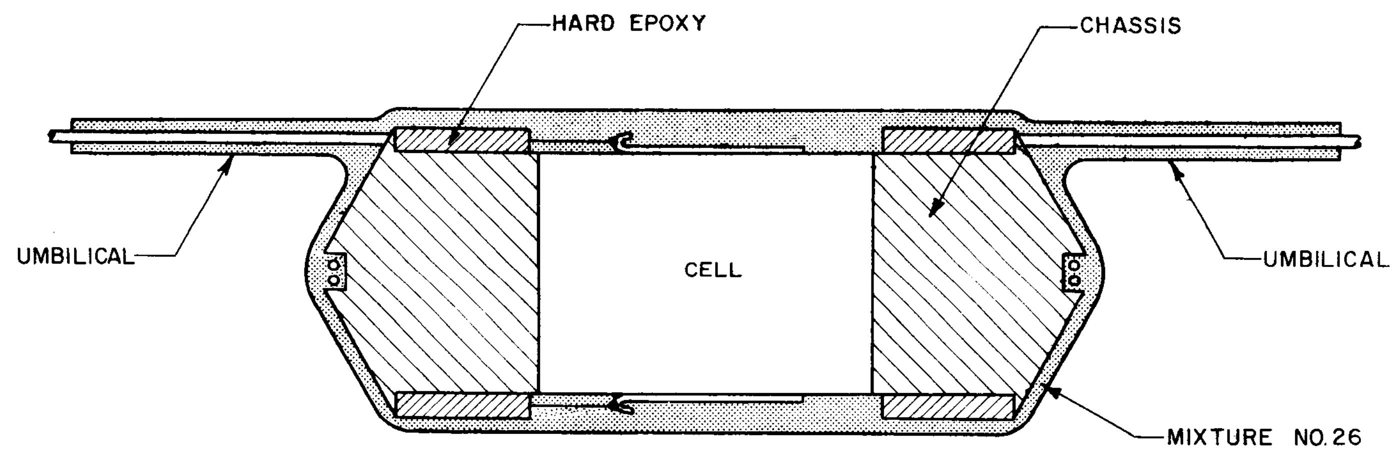


Fig. 4.3-11 - Encapsulation of MK V Telemeter.

Figs. 4.3-12 and 4.3-13 show the unit after the wiring decks have been filled with Sylgard.

Following the cure of the deck filling, the entire Mark V was coated with flexible Mixture 26. The finished unit is illustrated in Fig. 4.3-14. In this form, the unit weighed five grams and displaced a volume of $2\frac{1}{2}$ cc.

4.4 ELECTRICAL DESIGN, THE TELEMETER

4.4.1 MODULATORS

Justification for the use of an fm/fm system was discussed to some extent in our report F-B2299 (4-1). Our comments made in that report still hold.

Perusal of IRIG* Standard Modulation Frequencies results in convenient and useful information. For example, standard filters exist for the following frequencies and bandpasses:

(f_c), Center Frequency (Kc/s)	Bandpass (3db point)
0.4	$\pm$ 7.5% f_c, $\pm$ 15% f_c
0.56	
0.73	
0.96	
1.3	
1.7	
2.3	
3.0	
3.9	
5.4	
7.35	
10.5	
14.5	
22 etc.	

*Inter-Range Instrumentation Group

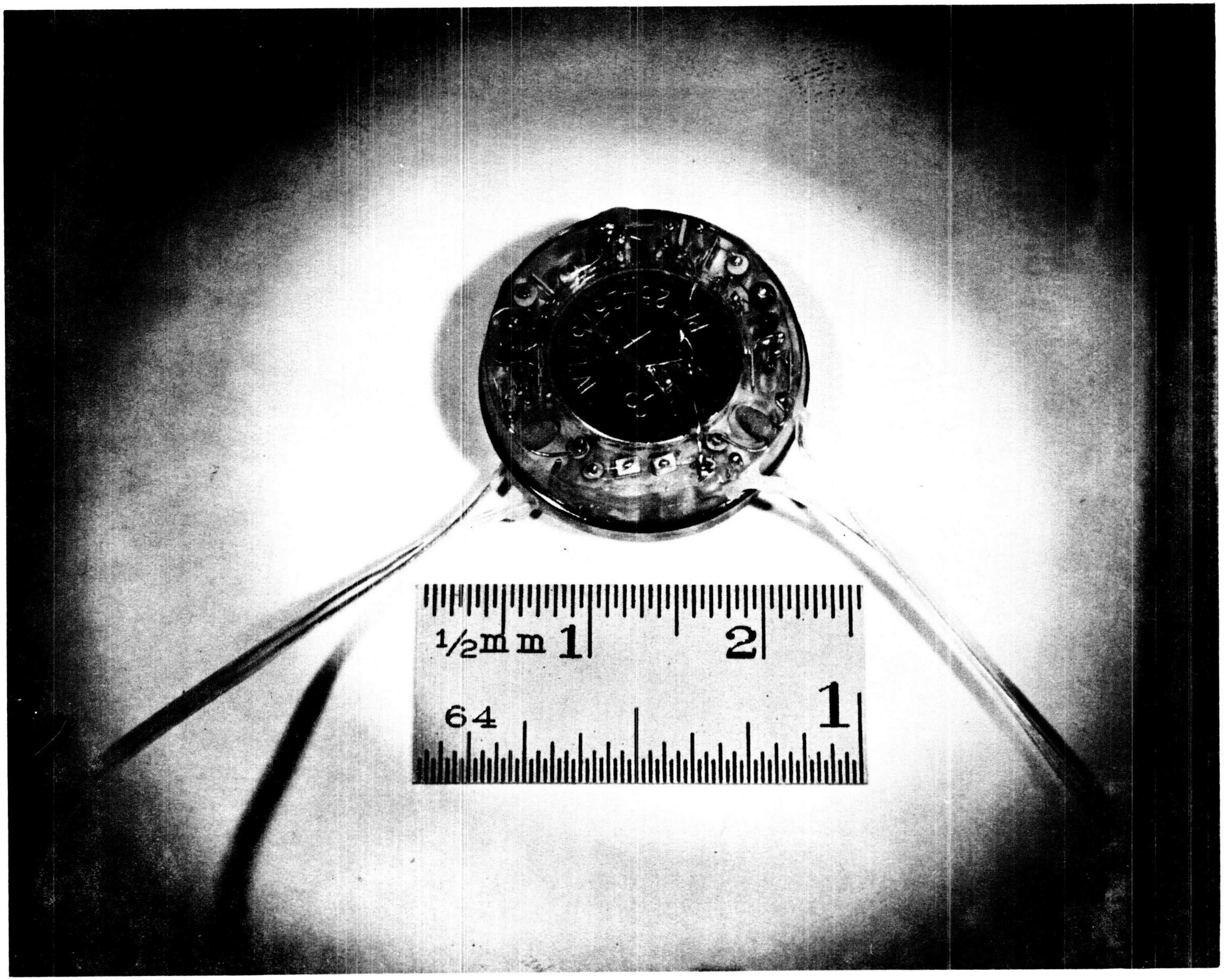


Fig. 4.3-12 - Mark V, Plastic Filled Deck (+ Side)

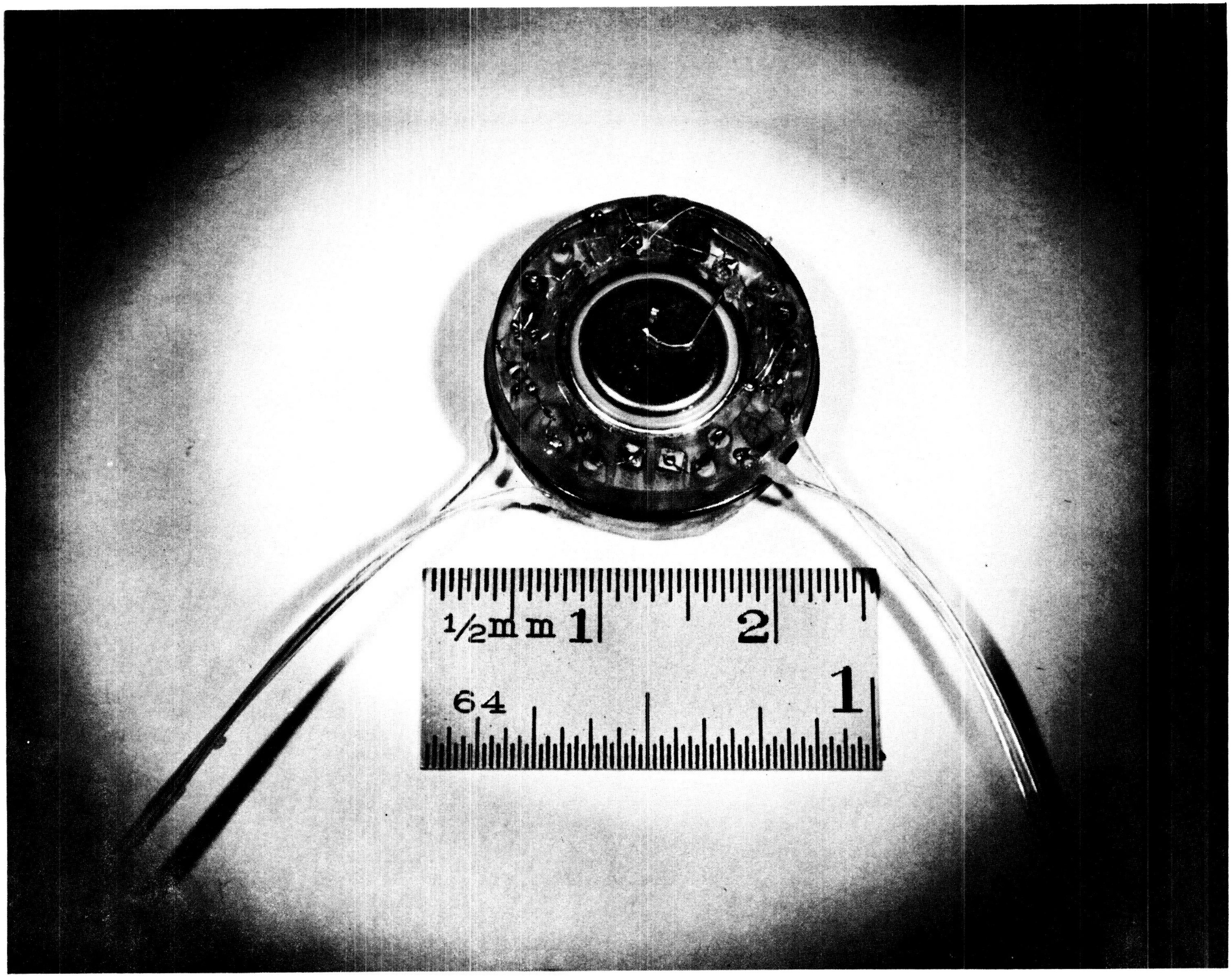


Fig. 4.3-13 - Mark V, Plastic Filled Deck (- Side)

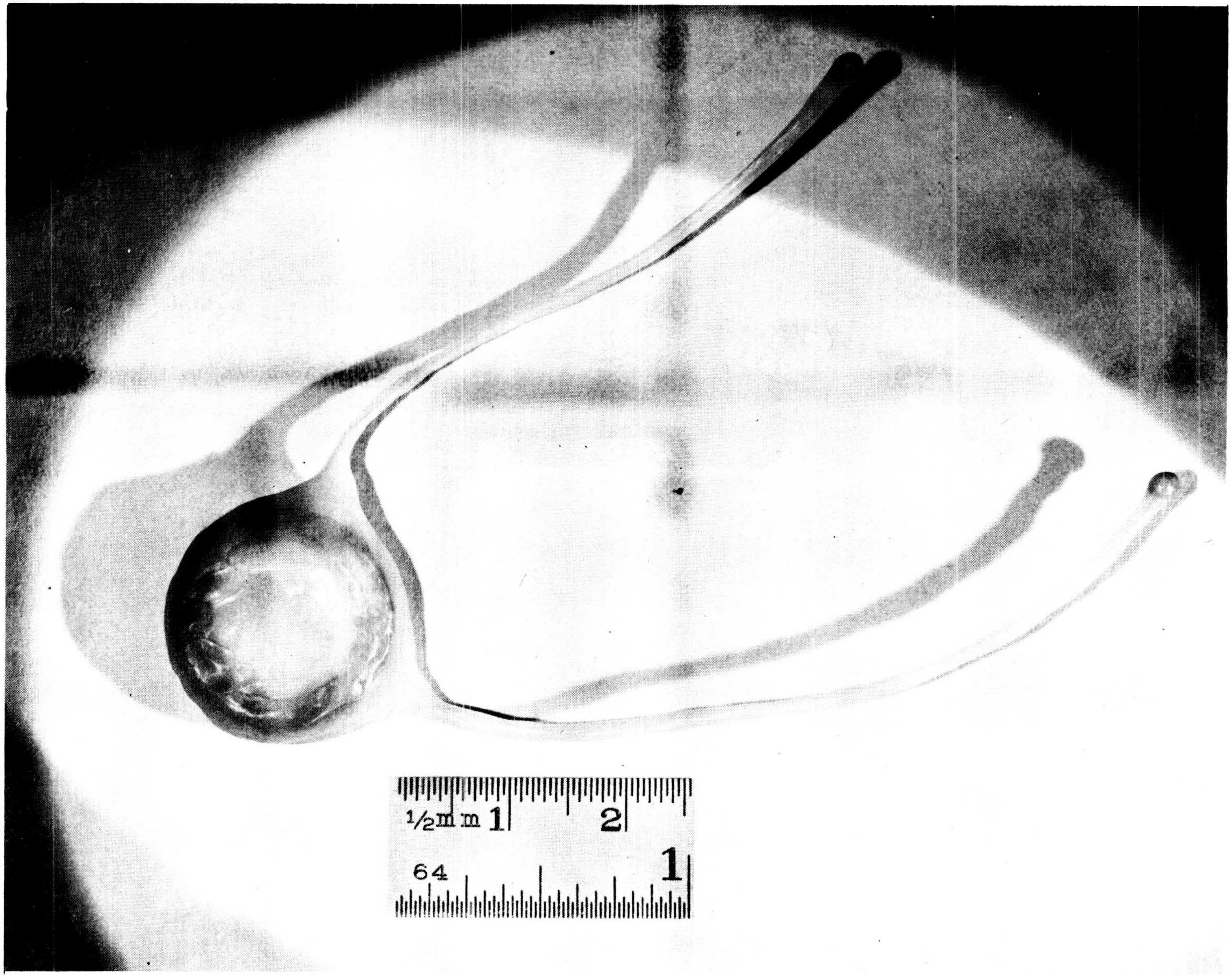


Fig. 4.3-14 - Mark V, Totally Encapsulated with Mixture 26

It follows then, that by utilizing IRIG standard frequencies, one may apply standard, commercially available, well-designed, filter elements in the decoding portion of the circuitry essential in the system receiver design. It is worth stressing at this point that total system design must contribute to specific telemeter design. This sort of approach affects equipment simplicity and cost—both are factors of interest to the biologist who will ultimately apply the results of such developments. These matters will be discussed further in their implication in Section 4.5.1 of this report.

With regard to modulator design a number of questions had to be answered. For example, would there be a net improvement in telemeter performance if the transmitter itself were pulsed-on by the several channels of modulation? Would there be a net system improvement if the various modulators were sequentially sampled? What were the relative virtues of pulsed modulation versus sinusoidal modulation?

If one were to use a pulsed-on transmitter, the individual modulators would have to supply greater energy to the transmitting oscillator so that it could be driven out of a biased-off condition. If one were pursuing this course with a single channel of telemetry, there appears little question that a substantial conservation of battery energy would result. However, the situation is altered somewhat if a multiplicity of modulation channels are desired, and particularly if a reasonable number of telemeters are envisioned for a single laboratory. In this latter case, not only does modulator current begin to assume substantial proportions, but without question the bandwidth required for a pulsed-on unit is substantially greater than that required by the continuously oscillating unit. This affects adversely signal/noise considerations at the receivers.

With regard to sequential sampling of modulation channels, it was our feeling that the circuitry necessary for such operation would unduly complicate both the transmitter and the receiver.

Pulse modulation versus sinusoidal modulation posed a more interesting problem. In the first place, simple modulators, requiring extremely low power for operation, are more easily designed for pulsed than for sinusoidal operation. Secondly, a substantial net saving in modulation power was anticipated by utilizing a pulse system—and particularly one based on a complementary (NPN-PNP) semiconductor design.

Based on these observations then we designed several pulsed modulators based on the complementary astable flip-flop. It became quite clear on attacking the modulator circuit design that the circuit itself was somewhat sensitive to temperature. This effect can hardly be considered surprising in view of the fact that total average circuit drains are on the order of 2 to 3 x 10^{-6} amperes. The effect is, however, serious in its detailed consequence to potential biological applications, a matter particularly important when the biologist desires to use a highly localized sensor remote from the telemeter body itself. This matter is important because in these latter circumstances the telemeter body can conceivably rest at a temperature <u>one or more $^{\circ}$F different</u> from that being experienced at the tip of the umbilical (the location of the sensor).

It thus became mandatory that the modulators be temperature-stabilized over a range of at least $\pm$ 2°C different from the remote sensor. This problem was interesting in that its solution had to be accomplished at an absolute minimal cost in current drain and with the smallest possible number of components.

After some study of the basic circuit which is illustrated in Fig. 4.4-1, two important facts became clear. First, the pulse-repetition frequency (prf) of this circuit is related to its applied voltage and second, its prf is related to temperature. Fig. 4.4-2 is a rough representation of these relationships. It was clear then, that if a passive network could be designed to vary the applied circuit voltage in a manner to offset the variation introduced by temperature, stability would result.

Such an effort was undertaken, and the results are illustrated in Figs. 4.4-3, 4.4-4 and 4.4-5. The measurements necessary to achieve these data were made by using a high-speed, stable oven for control of the modulator ambient temperature and the insertion

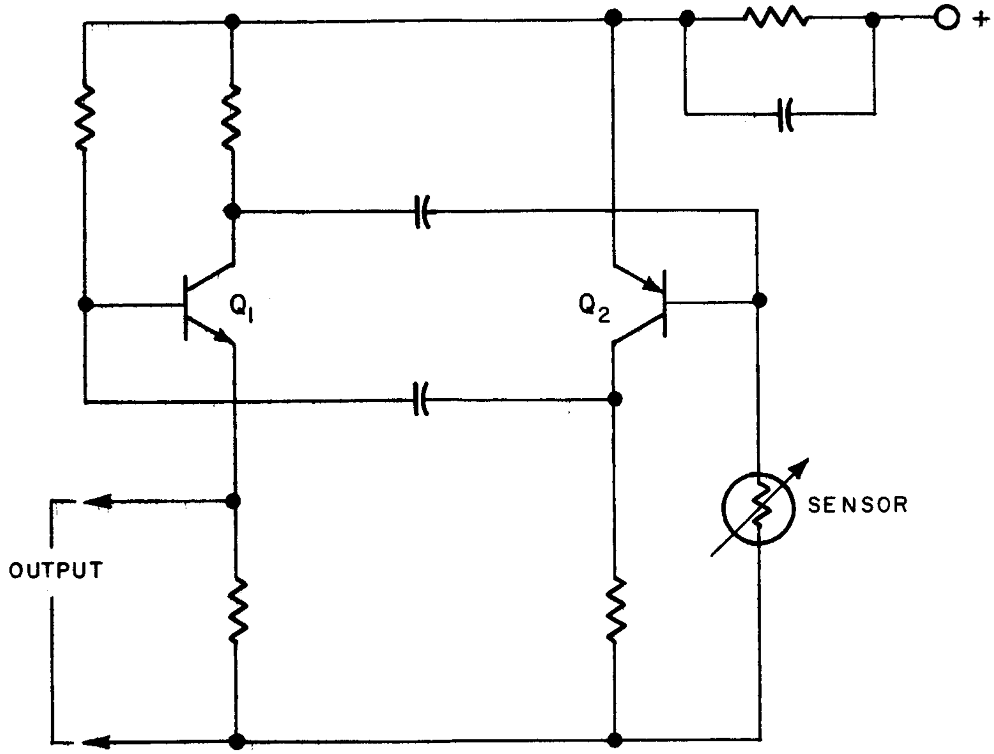


Fig. 4.4-1 - Basic Modulator Circuit.

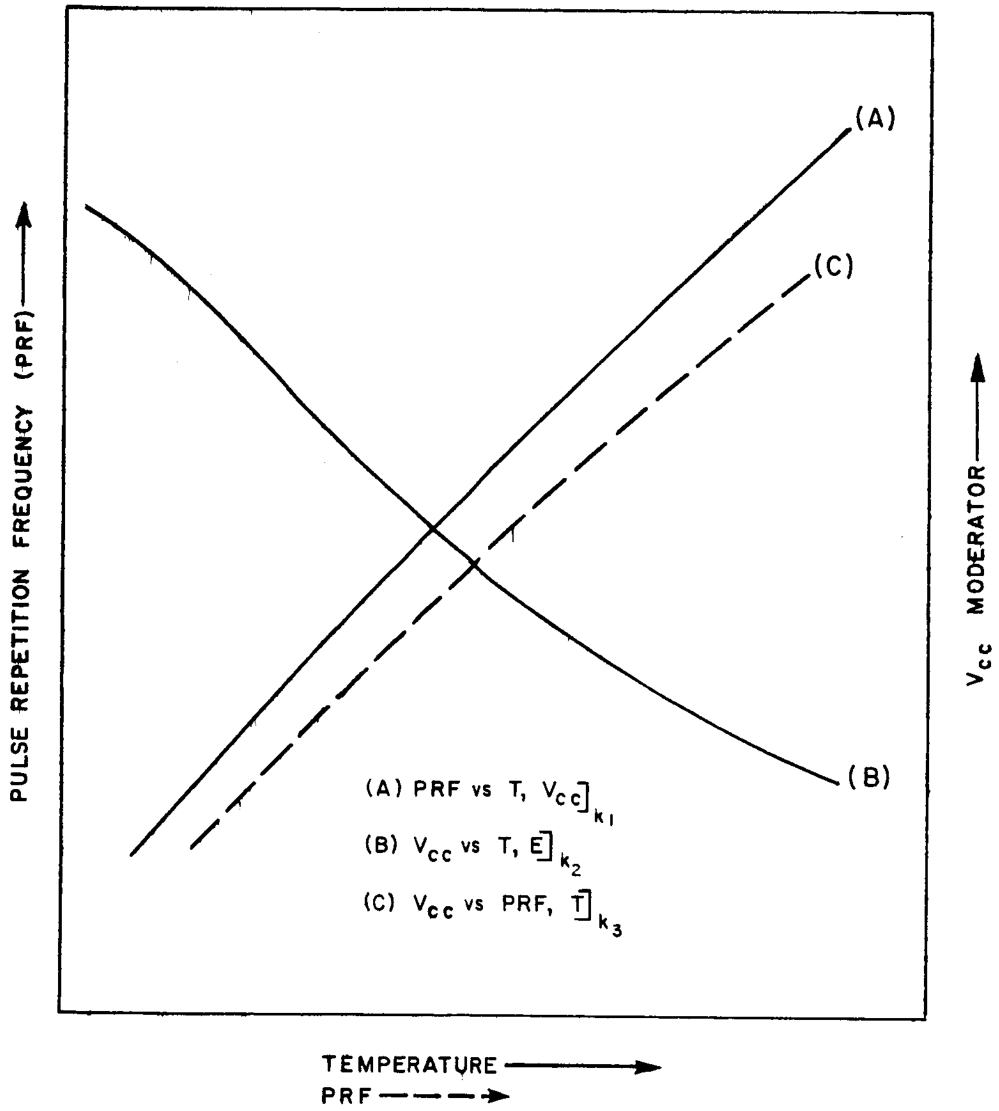


Fig. 4.4-2 - Modulator Circuit Characteristics

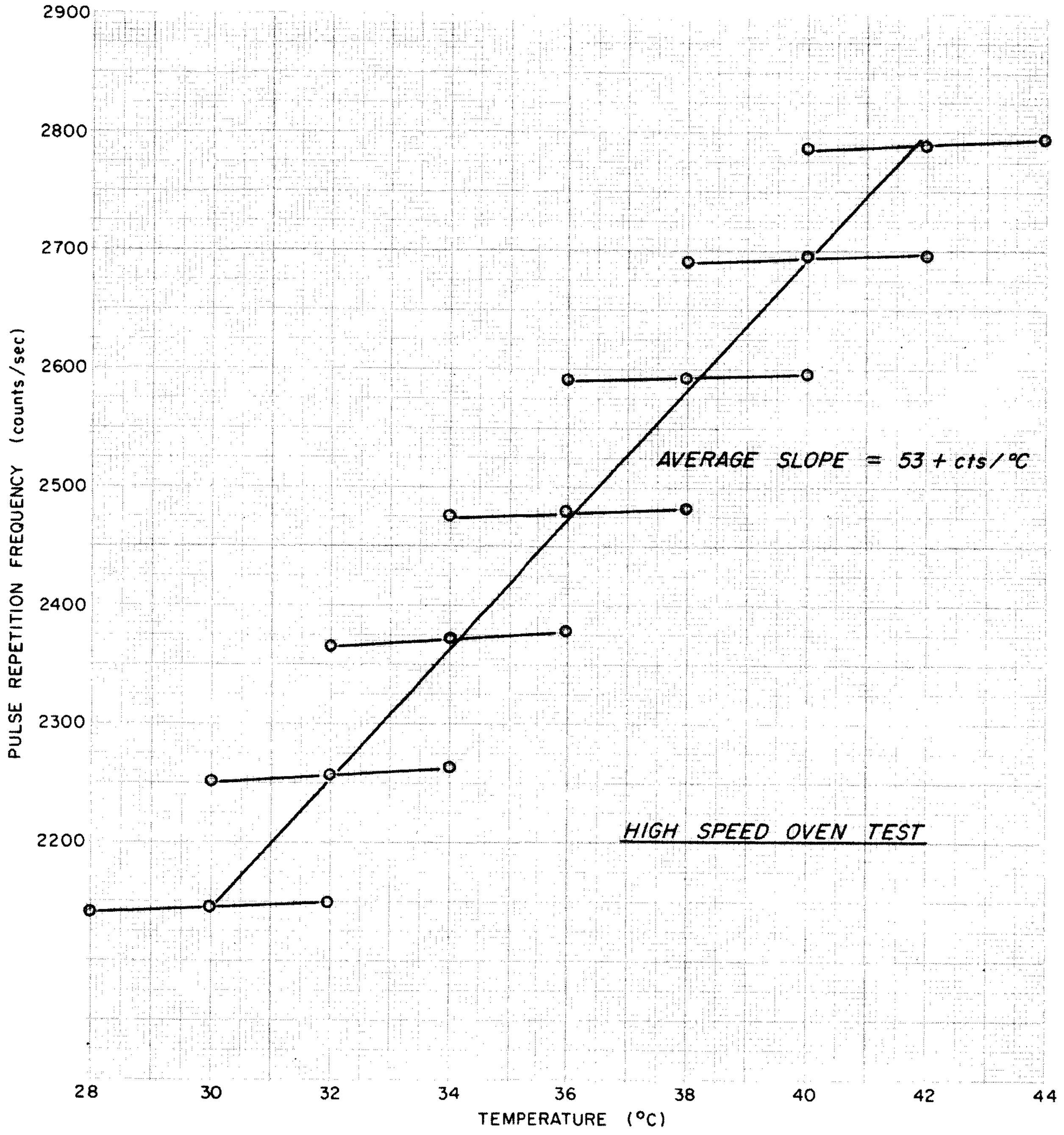


Fig. 4.4-3 - Modulator Response After Stabilization, Ch-1.

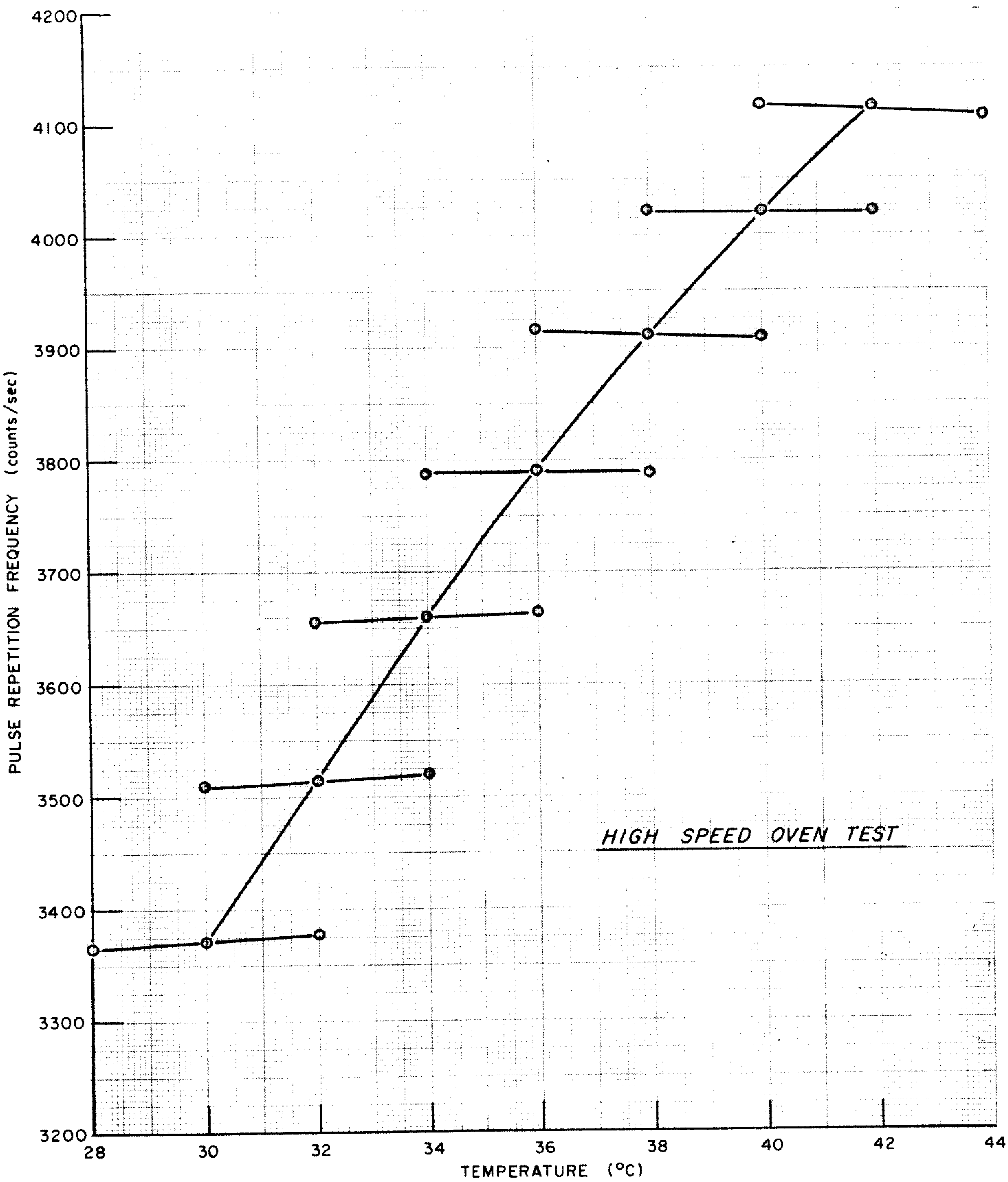


Fig. 4.4-4 - Modulator Response After Stabilization, Ch-2.

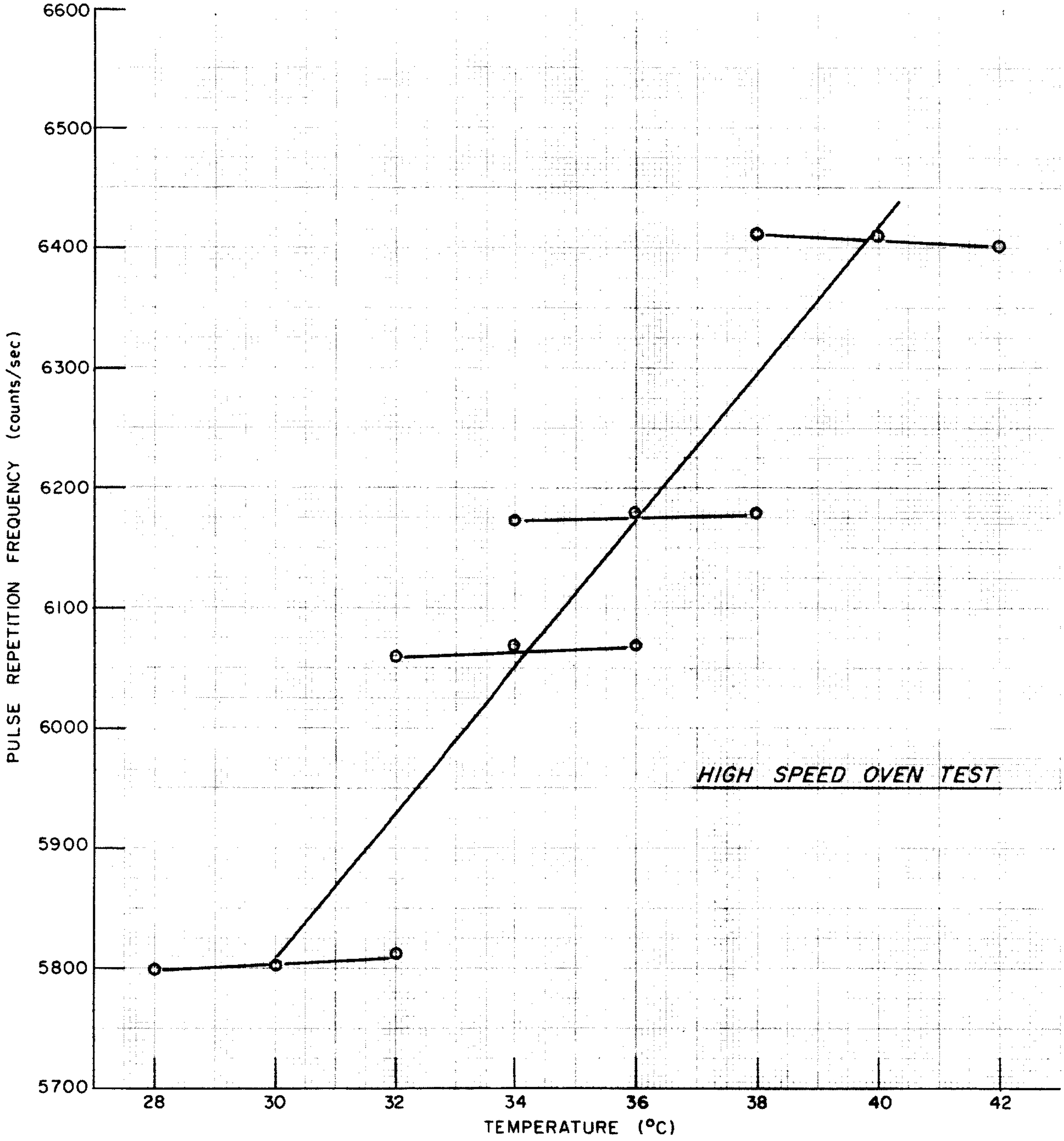


Fig. 4.4-5 - Modulator Response After Stabilization, Ch-3.

of theoretical sensor temperature by use of accurate representations for the pertinent thermistor sensor resistance value.

The figures mentioned above are interpreted as follows: The single curve plotted for pulse-repetition-frequency versus temperature is the total circuit response including the sensor. The horizontal curves, which appear at intervals along the circuit-response-curve, represent repetition frequency response for the total circuit when the remote sensor is held at a fixed temperature indicated by the crossover of the horizontal curve and the circuit response curve—and the circuit itself is shifted over the temperature range indicated by the horizontal curve. The temperature excursions used in the laboratory study were $\pm 4^{o}C$ or almost $\pm 8^{o}F$, more than sufficient for biological problems of the sort discussed above. Actually, stabilization over the differential range of $\pm 1^{o}C$ should normally be adequate.

In the past, most telemeters for biological application have used sensors embedded in the same mass as the implant circuitry. This arrangement generally obviated the need for such stabilization as is described above. The unwary may be tempted to calibrate telemeters with umbilicals in toto in a water bath—not suspecting the mayhem which could result if a temperature differential existed between the remote sensor and the telemeter body when the unit is implanted. We know of no simple way to avoid the problem other than by circuit stabilization. Fig. 4.4-6 illustrates the resultant circuit.

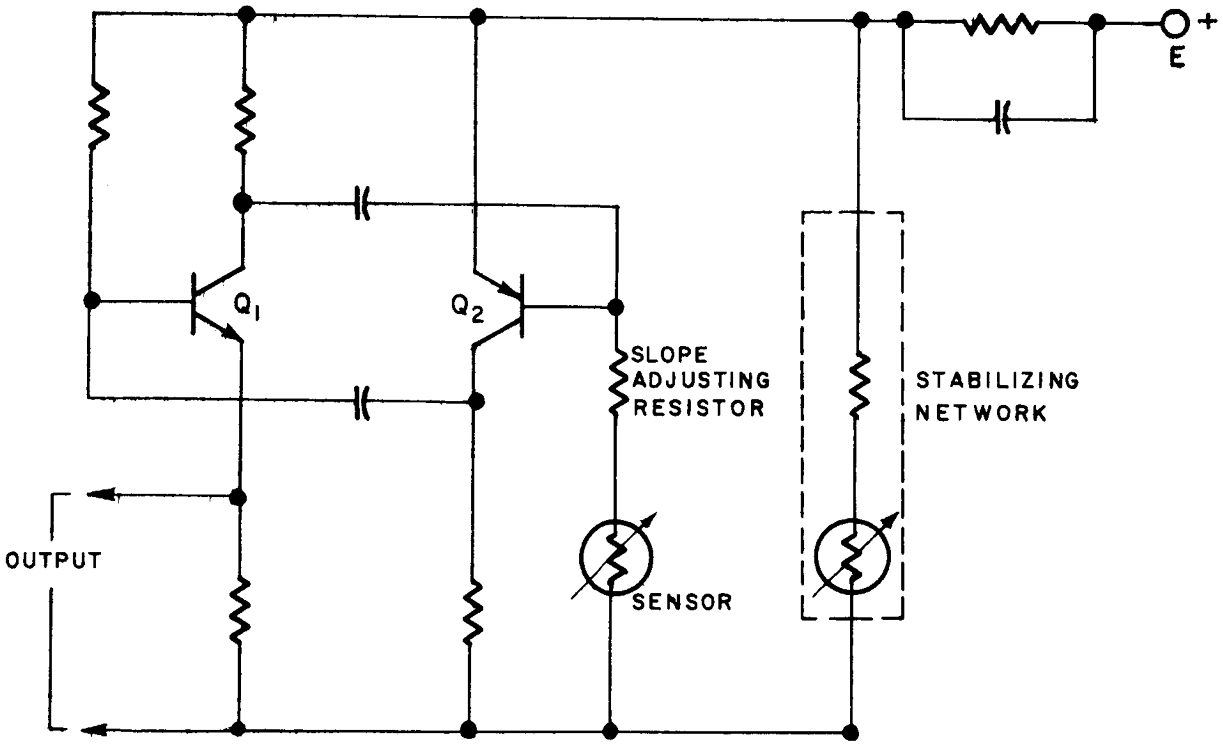


Fig. 4.4-6 - Circuit, Temperature Stabilized Modulator.

Measured current drain for the modulator type illustrated (including stabilization) is approximately 2.5×10^{-6} amperes.

The modulator output is a pulse of about 20×10^{-6} seconds in duration. At an f_c of 2.3 Kc/S, the duty cycle for channel 1 is 4.6% and for $f_c = 3.9$ Kc/S; the duty cycle is 7.7%. These percentages can be somewhat improved but are really not of serious consequence in overall energy use.

4.4.2 THE TRANSMITTER

The final transmitter design (including modulators) used in this first Mark V telemeter is illustrated in Fig. 4.4-7.

Because of difference between oscillator transistors, continuous and stable oscillation is obtained by adjusting R_1. In the Mark V/2 (T,T), SN-1, total oscillator current is 37×10^{-6} amperes.

This circuit uses the type FSP-411-1 transistor. It has been indicated by some researchers that the 2N918 gives better low current performance than the FSP unit. This report is, however, contradicted by others. Other approaches to solving the transmitter problem are in hand and will be discussed in a later section of this report.

A variation in signal strength and directivity was noted between situations wherein the telemeter was in "free space" and when it was immersed in a 20-gallon water tank.

This variation in radiated field is a matter of considerable interest to the biologist. For example, we feel quite certain

NOTES

MODULATORS ARE WELL ISOLATED FROM POWER SOURCE AND FROM EACH OTHER.

A THESE SENSORS ARE LOCATED AT THE DISTAL ENDS OF UMBILICALS FOR LOCALLIZED TEMPERATURE SENSING.

B THESE SENSORS ARE LOCATED WITH THE CIRCUIT BODY

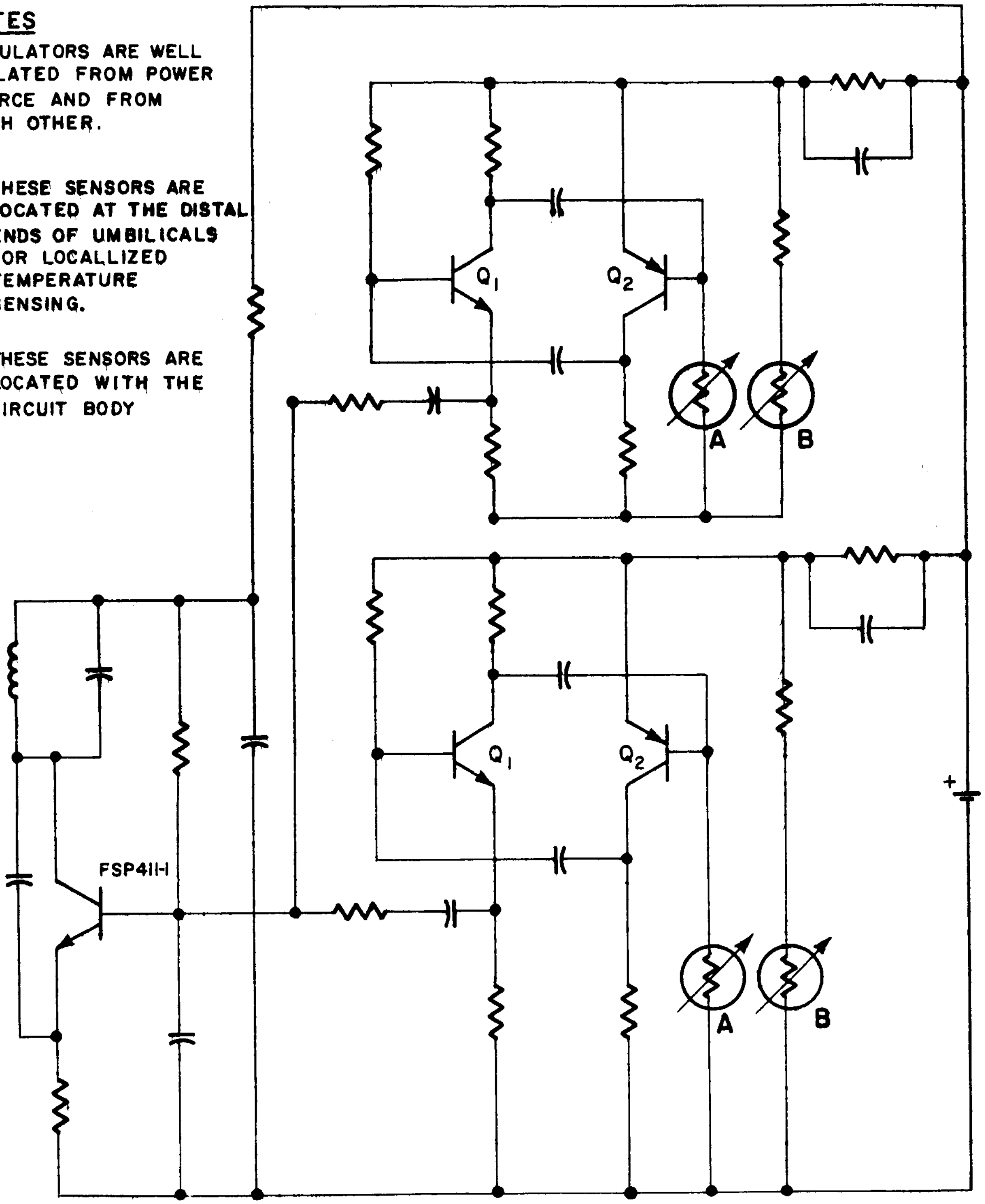


Fig. 4.4-7 - Circuit, Telemeter Transmitter Complete.

that the radiated field pattern—and to some extent its strength, is related to the size and shape of the animal containing the implant. This matter will be reported on further as we accumulate data.

This first unit proved to oscillate at a frequency of about 103 Mc/s; when it was deck-coated with Sylgard, the radiated frequency fell to 100 Mc/s; when total encapsulation was completed (flexible paraffin) the final frequency was 98 Mc/s. Upon submersion in the water bath, frequency was 97 + Mc/s. The latter minor variation is of interest.

4.4.3 TELEMETER PERFORMANCE

Final measurements on total telemeter response time to temperature step at the sensor are not yet in hand. We anticipate response time constants on the order of three to five seconds.

Fig. 4.4-8 illustrates transmitted response to water bath temperatures for both channels of telemeter transmission. We are not quite as pleased with Channel 2 as with Channel 1. However, we believe both channels will prove extremely useful.

We note that Channel 1 has a slope of 55 counts/^{o}C, a least count of .018^{o}C and that Channel 2 has a slope of 55 counts/^{o}C, a least count of .018^{o}C. During water-bath calibration we were pleased with the excellent stability of the unit at constant temperature.

Telemeter lifetime is computed at approximately five 30-day months.

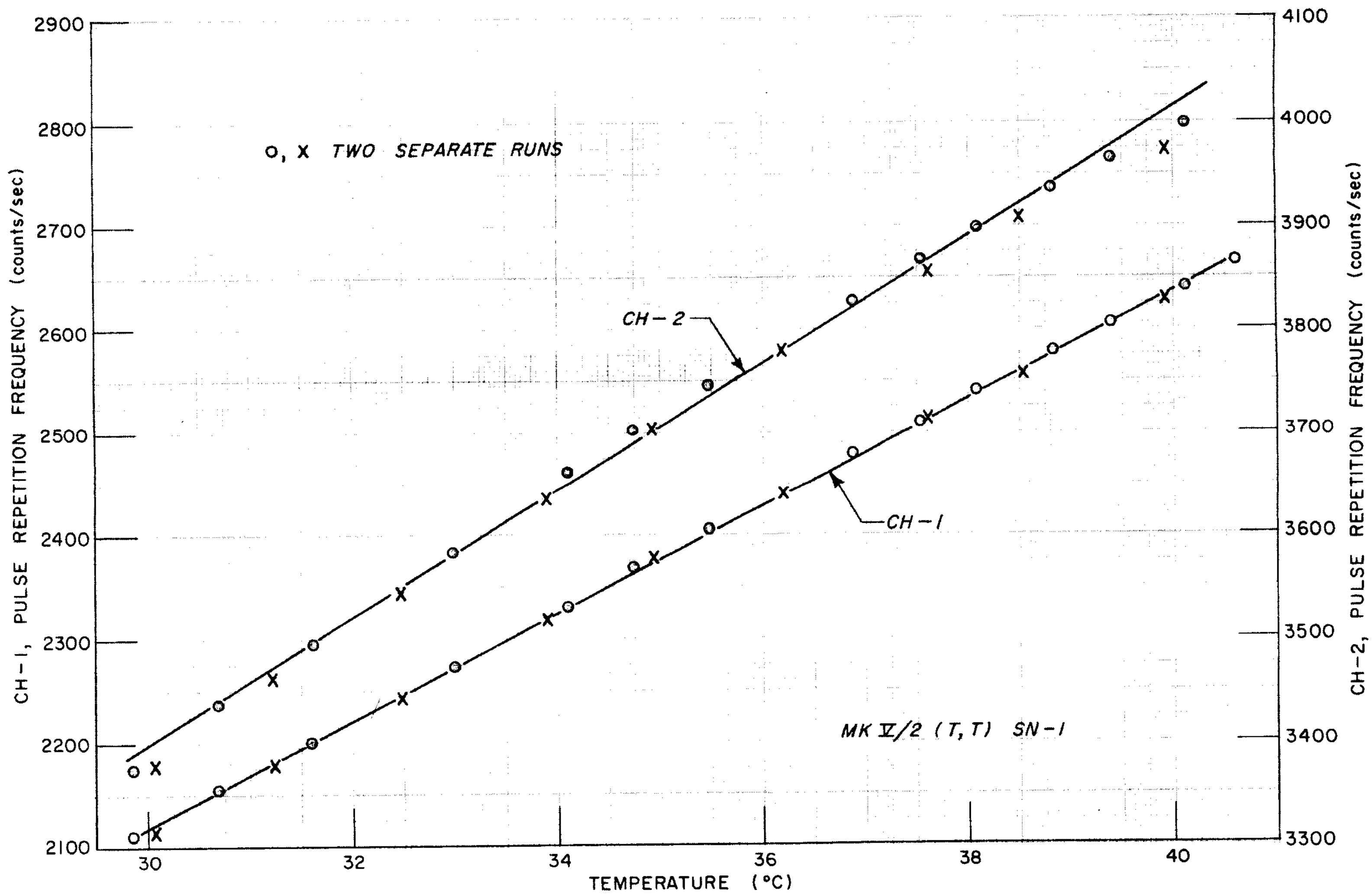


Fig. 4.4-8 - Response Curves, MK V, Channels 1 & 2.

4.5 THE RECEIVER SYSTEM

4.5.1 MODULATION AND THE RECEIVER DESIGN

The reader will recall that in the preceeding section (4.4.1) of this report, we mentioned the several advantages of pulsed modulators. Of particular interest was the very low energy requirement for such modulators.

Having determined on pulse-type modulation we now turn to the design of the related receiving system. The system desired was to be relatively inexpensive, straightforward in operation and capable of adequate separation of transmitted channel data.

Our initial approach to the design led us to a consideration of the so-called "dead-time" gated receiver. Such systems are intriguing because of their reasonable simplicity plus a substantial improvement in signal-to-noise (S/N) ratio. If one were considering the reception of a single channel of data, and if the prf range for the data were (say) 2000 to 2500 pps, then a simple receiver system as illustrated in Fig. 4.5-1 could be utilized. In such a system, every modulation pulse triggers the one-shot circuit and that circuit generates an inhibiting signal which "kills" the FM receiver for a period of time equal to

$$\frac{1}{f_{max}} - \Delta t,$$

where f_{max} = maximum prf

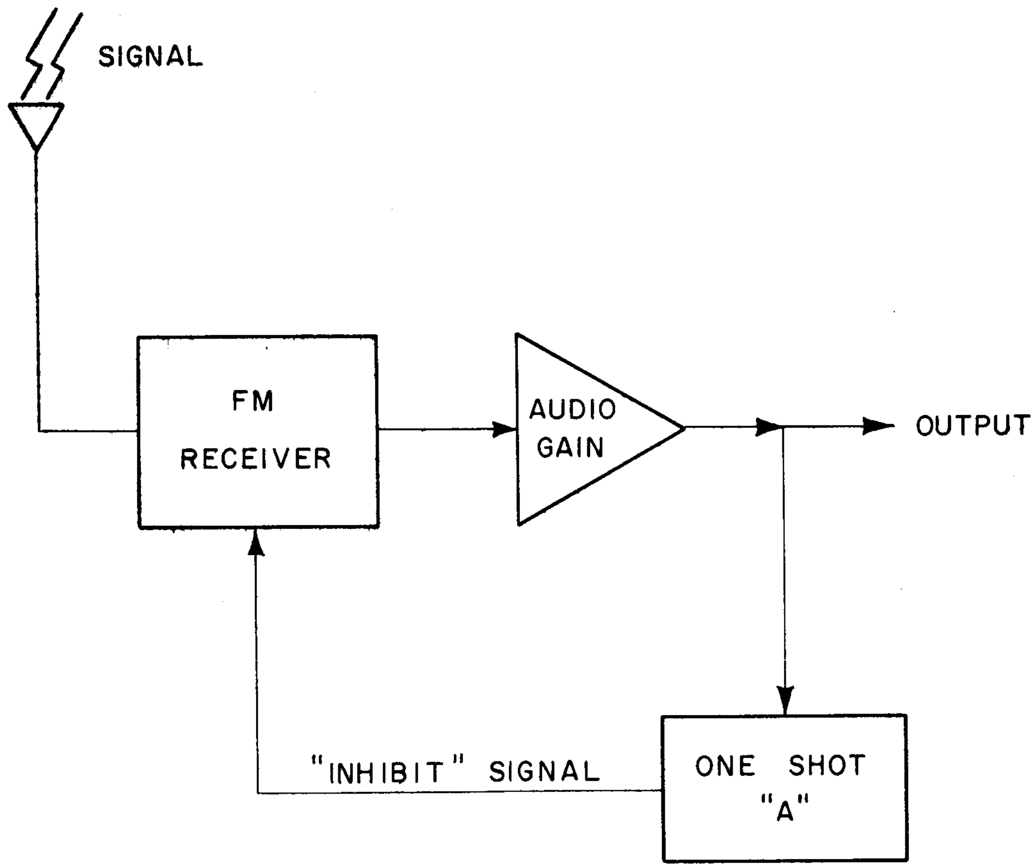


Fig. 4.5-1 - Single Channel "Dead-Time" Gated Receiver

Δt = time increment to preclude possible exclusion of maximum pulse rate

Therefore, using the prf range suggested above, the dead-time for the receiver will be 0.4 ms - Δt. This is an attractive circuit from several points of view. First, it is simple and second, since we know that no signal pulse can possibly occur during the inhibited period we have materially improved the signal-to-noise ratio of the entire system. If the prf is at the low end of the suggested range (2000 pps), we lose a small part of the advantage of this arrangement because the receiver becomes uninhibited 0.1 ms + Δt before the next modulation pulse arrives. In a gross way our S/N improvement is thus degraded by about 25% at the low end of the modulation frequency range. Systems such as this have been installed by us on the Franklin-Princeton Data Acquisition Unit (Moffett Laboratory) and have been used with success in Rhesus monkey studies.

Now let us consider a multiplicity of modulation channels, that is to say, more than one. Consider the two we are using:

Channel 1 with f_{c1} = 2.3 Kc/S

Channel 2 with f_{c2} = 3.9 Kc/S

Reference to Fig. 4.5-2 illustrates a possible system. The inhibiting or blanking pulses from each one-shot circuit (A and B) can be tailored for the prf ranges to which they are indicated to be related. Unfortunately, however, we note that the Channel 2 chain of the system is susceptible to all frequencies above 3 Kc/S.

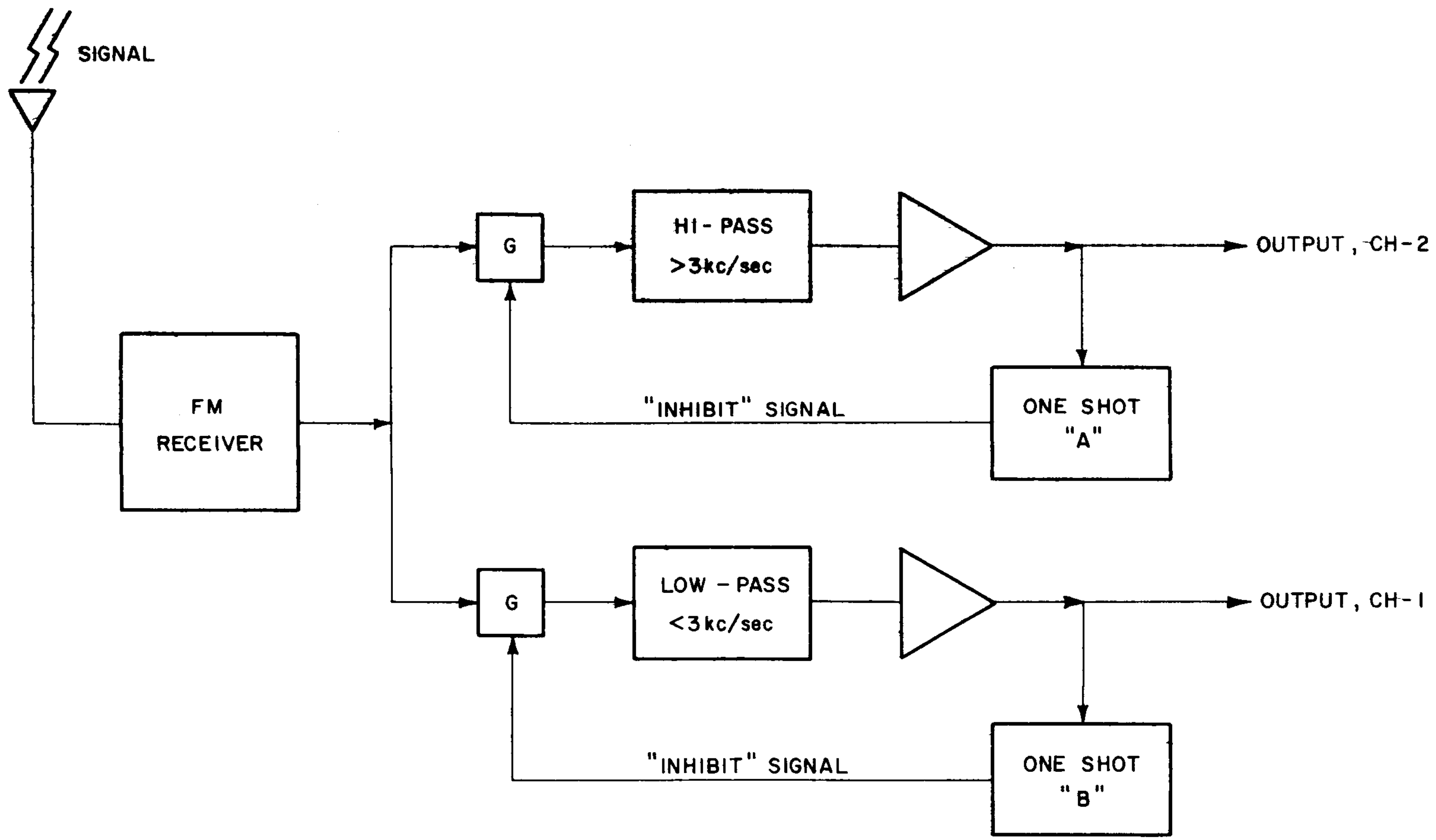


Fig. 4.5-2 - Dual Channel "Dead-Time" Gated Receiver

Note that the pass-filters illustrated would not normally be included in a straight dead-time circuit. Their inclusion here represents an improvement in isolation between the two channels.

Further, we must add the observation that the telemeter modulators are not synchronous with respect to each other. As a matter of fact, they are extremely well isolated from each other so that malfunction in one modulator will have a negligible effect on the other. However, this lack of synchronization means that received repetition frequencies may be expressed as follows:

$f_{c1} \pm \Delta f_1$, channel 1

$f_{c2} \pm \Delta f_2$, channel 2

$f_{c1} + f_{c2} \pm \Delta f_1 \pm \Delta f_2$ Receiver Output

It becomes clear that if the two channels are modulated at frequencies substantially separated from each other and are not multiples of each other, then a two-channel system as illustrated can be made to work and to work with a resultant improvement in signal-to-noise ratio. Clearly, based on the simplified discussion above, the situation becomes rapidly more complex as the number of data channels being transmitted increases from two. The situation is so constraining that it becomes difficult to use standard IRIG channels, and modulator center frequencies spread rapidly over a range which may exceed the normal capability of standard broadcast FM receivers. We do not wish to be limited by such constraints—

in part because we plan three channels of data transmission and in part because we feel strongly that economical, commercially available receiving equipment must be applicable to our system.

We then turned to a more conventional approach for the receiver system design. Fig. 4.5-3 illustrates a generalized diagram for a receiver capable of multi-channel signal separation (n-channels). In order to discuss this receiver system more fully, let us first review briefly some fundamental characteristics of the pulse modulation itself(4-4).

Fig. 4.5-4a illustrates the generalized pulse modulation signal.

δ = pulse duration in seconds

$\tau_r = \frac{1}{f_r}$

f_r = pulse repetition frequency (prf)

Fig. 4.5-4b shows the relative amplitudes for the k th harmonic voltages arising from the pulsed signal. The relationship describing this situation can be expressed as follows:

$$a_k = 2E_o \frac{\delta}{\tau_r} \left(\frac{\sin \pi \, kf_r \delta}{\pi \, kf_r \delta}\right)$$

where a_k = voltage amplitude for harmonic k whose frequency is the integer $k \times f_r$

δ = pulse width in seconds

τ_r = pulse separation in seconds

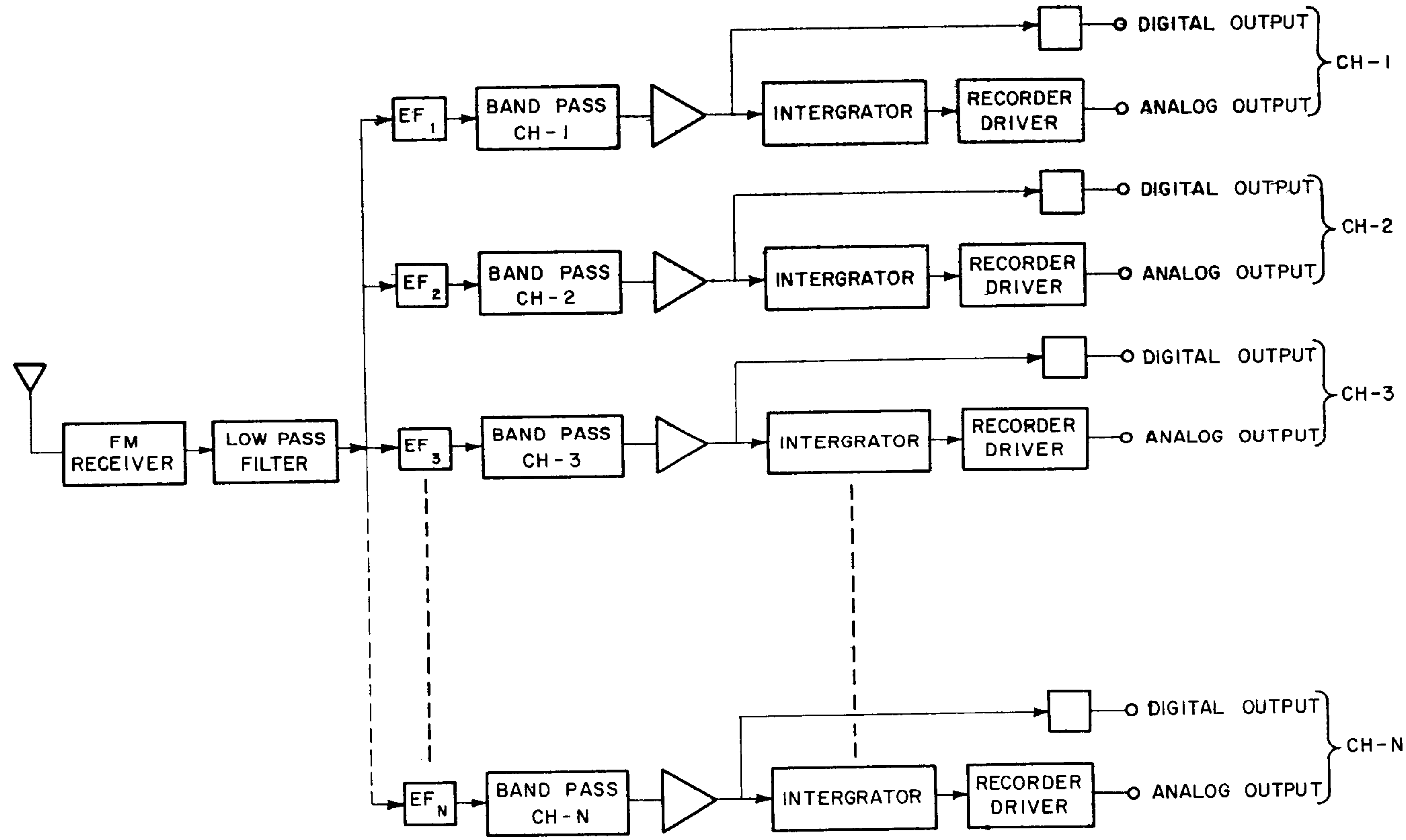
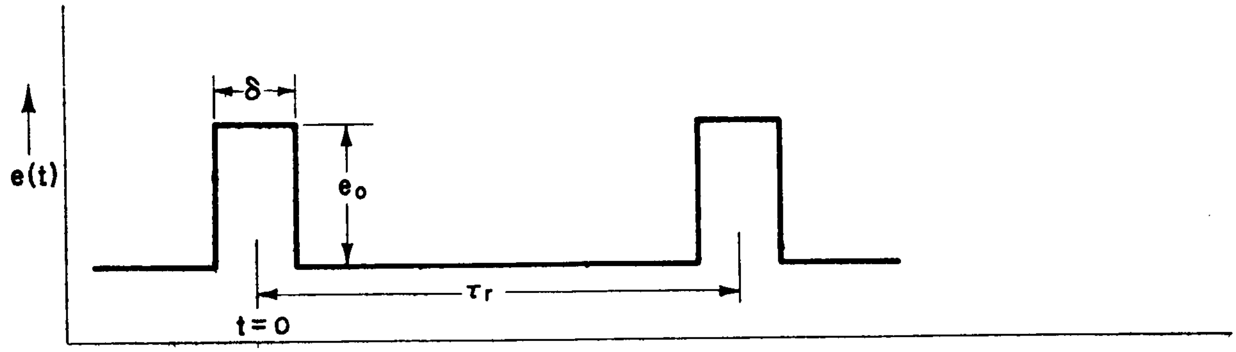
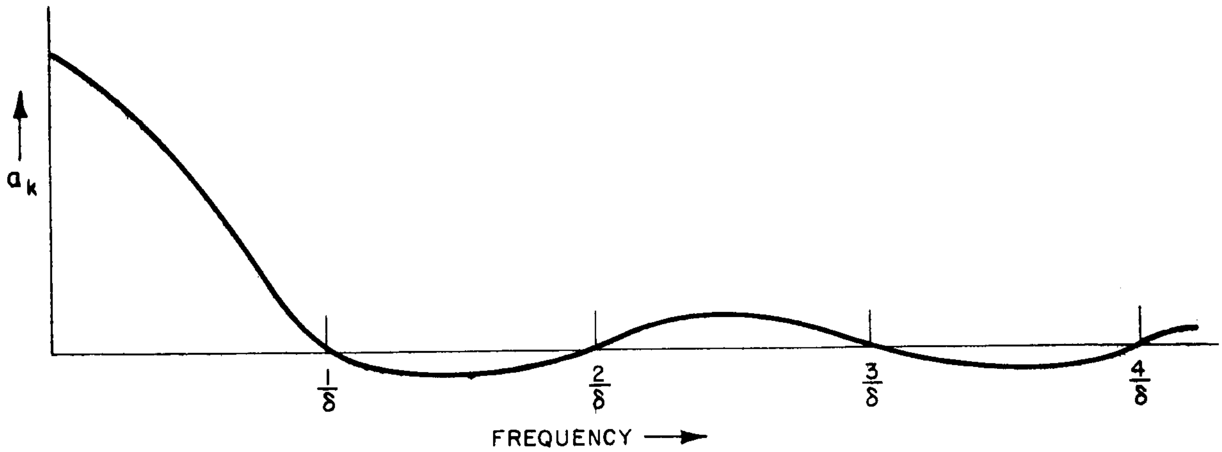


Fig. 4.5-3 - Multi-Channel FM Receiving System



a) Generalized Modulation Signal



b) Harmonic Voltage Amplitudes Versus Frequency

Fig. 4.5-4 - Modulation Characteristics

It is clear then that the available received modulation signal peaks very close to the pulse repetition frequency. The plot illustrated in Fig. 4.5-4b becomes even more meaningful if it were squared showing proportionality to modulation power. Further, examination of the equation stated above provides additional information relevant to our design. For example, the amplitude of a_k, where $k = 1$ is directly proportional to $\frac{\delta}{\tau_r}$. This means that for a given repetition frequency the pulse width must not be reduced without limit or we shall reduce received modulation power in proportion to the inverse square of the stated ratio. Reduced pulse width also means that bandwidth at the receiver must be increased to receive equal signal power. These critical facts must therefore be related to transmitter modulation design. They impose a limit on the pulse duty cycle so that while one may wish to reduce pulse duration for a net saving in transmitter modulator power requirement, that desire must be sensitive to the fact that excessive pulse-width reduction will introduce serious S/N problems at the receiver.

Now let us look at the practical situation in hand. Channels 1 and 2 of the first prototype, experimental Mark V are temperature channels. For Channel 1, $f_{c1} = 2.3$ Kc/S and for Channel 2, $f_{c2} = 3.9$ Kc/S. The response of commercially available IRIG filters for these center frequencies is illustrated in Fig. 4.5-5. We could have used an $f_c = 3.0$ Kc/S except that we desired considerable

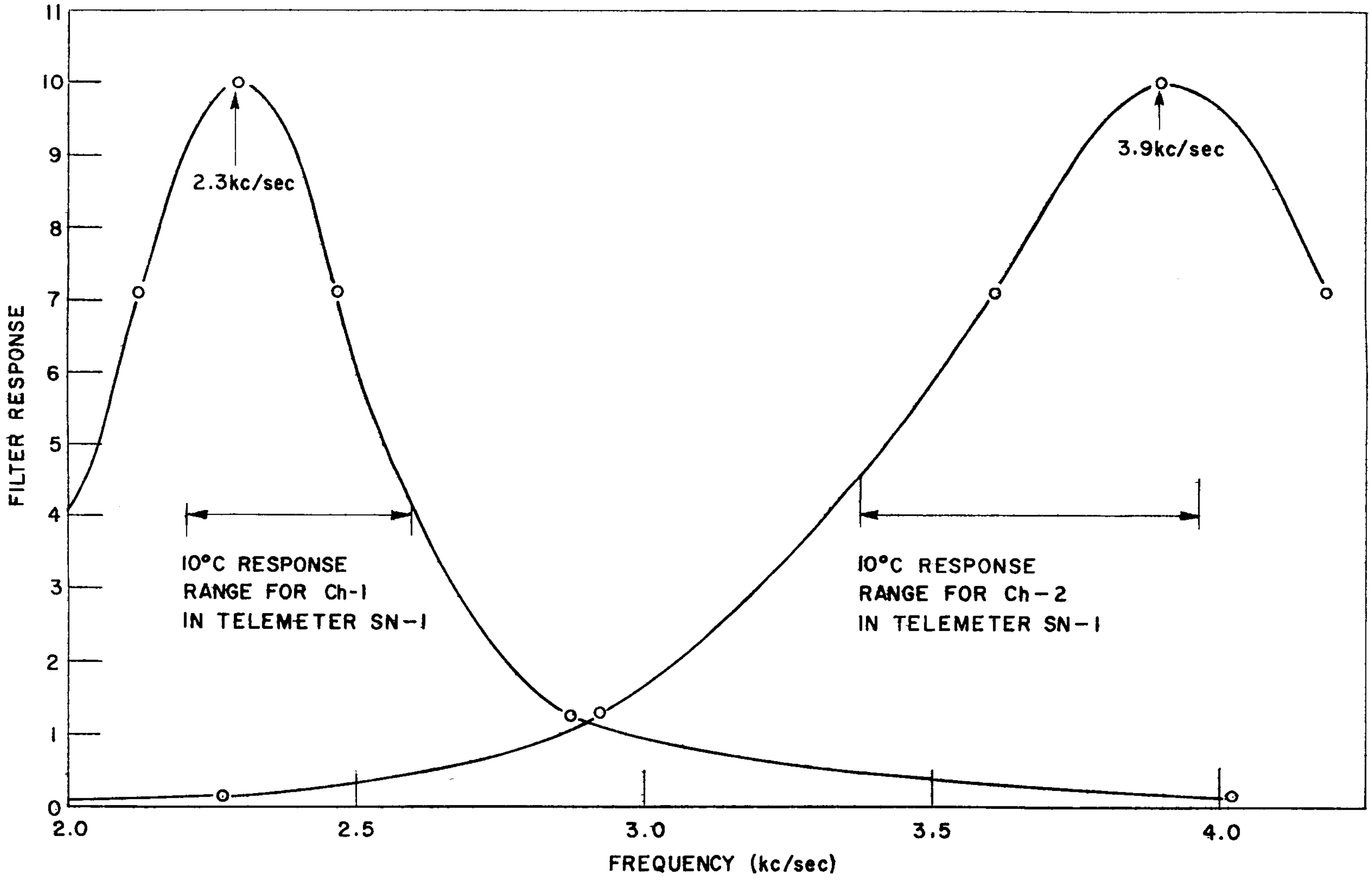


Fig. 4.5-5 - IRIG Filter Response vs Frequency

sensitivity in these temperature channels which requires a substantial frequency spread about the channel f_c. Such close spacing of the f_c's would then have presented more severe separation problems. Sum frequencies must also be considered as in the dead-time receiver. However, in the selection of f_c's this is not a difficult problem. The receiver design is illustrated in Fig. 4.5-6, which is a detailed representation of the block diagram of Fig. 4.5-3. In the detailed receiver the radio frequency signal is processed in an inexpensive AM/FM transistor radio ($19.00), detected there and amplified in the audio system of the receiver. The complex modulation signal is then processed through a low-pass filter and is separated into its channel components by the simple circuitry which follows the low-pass filter. Signal separation is quite good. Use of a more sophisticated (and more expensive) FM radio receiver simply improves our transmission distance capability for the system. Our use of the economical (cheap!) receiver was to prove a point: the biological researcher who will ultimately use the equipment now has the promise of an inexpensive receiving system for multi-channel telemeters.

The channel separation circuits work quite well and consume only 24×10^{-3} watts of power. It will be desirable for subsequent multi-channel receivers to incorporate integration circuitry so that transmitted waveforms can be recorded (as with ECG signals).

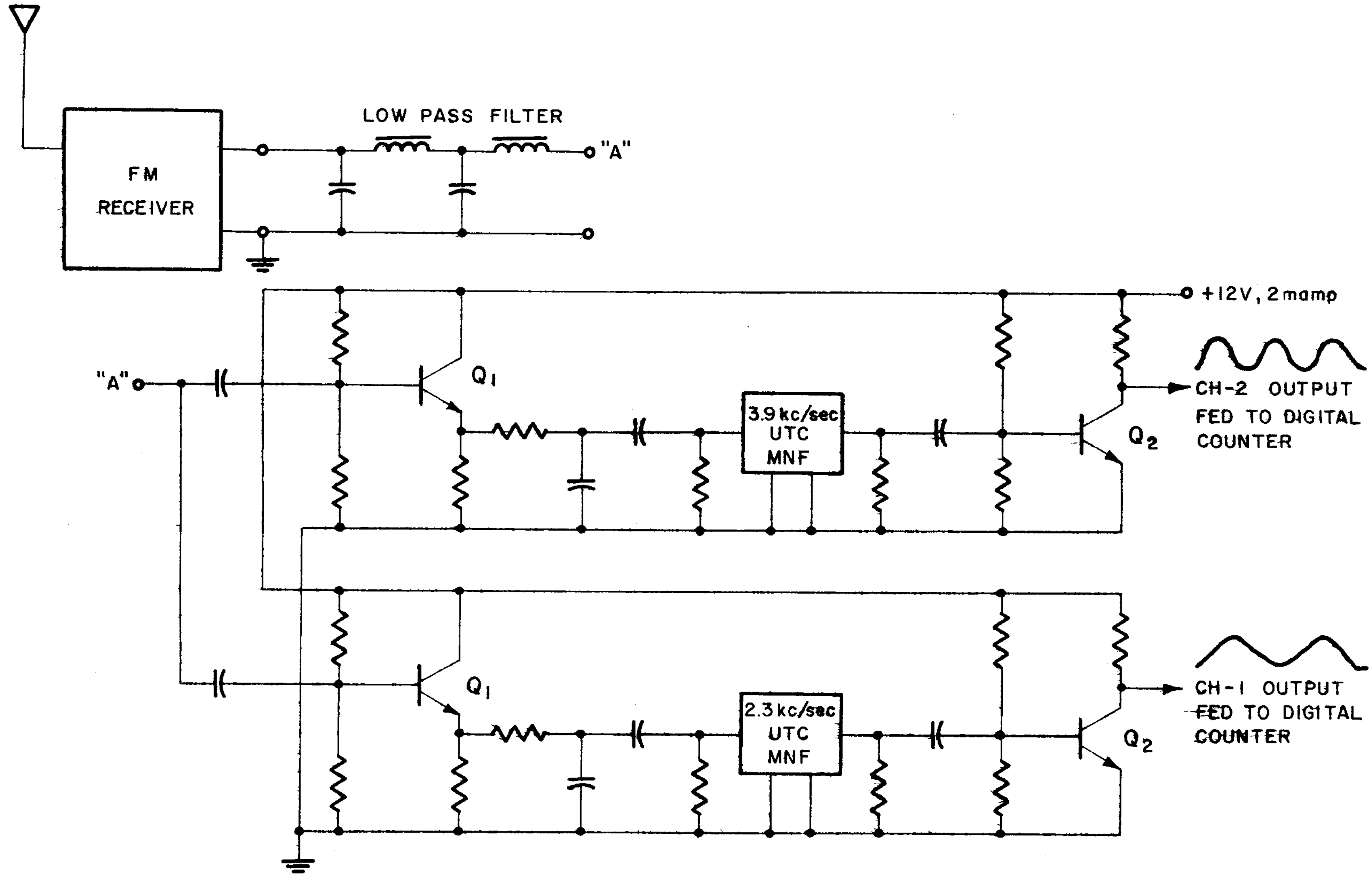


Fig. 4.5-6 - Dual Channel Receiver

No attempt has yet been made to package the receiver for general use. As we learn more about other data to be transmitted we can expect to standardize receiver design in such a manner that only the insertion of related f_c, IRIG filters will be necessary for proper operation.

To date, the only antennas used with the receiver have been simple rods. We anticipate the use of more sophisticated antennas in the future and may well expect considerable improvements in transmission range to accrue.

4.6 FUTURE PLANS AND PROBLEMS

Our next effort will concentrate on at least two telemeters. One will transmit temperature and ECG; the other will be designed for the simultaneous transmission of temperature, ECG (or ECG rate) and a "potential".

In the latter case, the potential mentioned will relate eventually to DC potential. The problems involved with this third channel are expected to be difficult to handle. No success has been reported in the literature to date regarding chronically implanted electrodes capable of sensing pH or dc biopotentials. It is certainly the case that quite apart from the design problems associated with micropower, microminiature circuits, we face the problem of sensor design. In the same light we will be interested in the design of pressure sensors which are very small and which will operate with precision as a chronic implant.

Transmitter power requirements will also be studied in an attempt to improve the efficiency of operation at substantially reduced current levels. The goal, of course, is to improve operational lifetime for the implant. New transistor types will be evaluted. as will be the relative merit of increased cell-voltage versus reduced transmitter current requirement.

We have also become extremely curious about the radiation patterns which result when VHF radiation sources such as Mark V implants are placed in moderately conducting media. Modifications in directionality were observed when the Mark V was placed in a 20 gallon water bath. We wish to know more about the loading effects on the transmitter itself, the possibility of predicting final radiation patterns from implanted units—possibly as a function of the size of the subject animal as well as of its shape, etc. Such a study may prove to be beyond the scope of our present time and funding, but in any event an understanding of these phenomena and their potential effects on the design of telemetric implants and receiving antenna systems is of fundamental importance.

4.7 REFERENCES

(4-1) Interim Annual Report F-B2299, Vol. II, Sections 4 and 5; 9 Nov. 1964 to 31 March 1966.

(4-2) Personal Communication, dated 28 December 1966

(4-3) Personal Communication, dated 23 November 1966

(4-4) Principles of Radar, MIT Radar School Staff, McGraw Hill Publishing Company, New York

APPENDIX 1

APPENDIX 1

DISCUSSION OF CRITERION MEASURE DIFFERENCE ANGLE

For analysis where $(Al-Al_{avg})$ or $Mu-Mu_{avg})$ are used as the criterion measure, let the angles under consideration be defined as follows:

A = total angle

H = component due to magnetic field

B = bias component due to apparatus

L = lunar component

Then we have four directions—each component may have a different value for each direction

let 1, 2, 3, 4 indicate the four directions

then, in general

$A_i = H_i + B_i + L_i$ for each value of i

let

$$A_1 + A_2 + A_3 + A_4 = \overline{A}$$

$$B_1 + B_2 + B_3 + B_4 = \overline{B}$$

$$H_1 + H_2 + H_3 + H_4 = \overline{H}$$

$$L_1 + L_2 + L_3 + L_4 = \overline{L}$$

Forming $\Delta A_i = A_i - \overline{A}$

$$\Delta A_i = H_i + B_i + L_i - (\tfrac{1}{4}\Sigma H_i + \tfrac{1}{4}\Sigma B_i + \tfrac{1}{4} L_i)$$

What we want is $\Delta A_i = H_i$

Therefore for this to be true (and assuming no interactions or correlation between H_i, B_i and L_i) that is assuming H_i, B_i and L_i to be independent then

1) $\frac{1}{4}\Sigma H_i = 0$
2) $B_i - \frac{1}{4}\Sigma B_i = 0$
3) $L_i - \frac{1}{4}\Sigma L_i = 0$

For condition 1) to be true we can have several simple sets of circumstances:

a) all H_i may be 0

or b)

$$\left.\begin{aligned} H_1 + H_2 &= H_3 + H_4 \\ H_1 + H_3 &= H_2 + H_4 \\ H_1 + H_4 &= H_2 + H_3 \end{aligned}\right\} \text{Sum of any two H's = sum of other two H's}$$

$$\left.\begin{aligned} H_1 &= H_2 + H_3 + H_4 \\ H_2 &= H_1 + H_3 + H_4 \\ H_3 &= H_1 + H_2 + H_4 \\ H_4 &= H_2 + H_3 + H_4 \end{aligned}\right\} \text{One H = sum of other three H's}$$

or c) some H_i's may be zero and the sum of the remainder equal zero

Magnitude as well as direction of effect determine whether $\frac{1}{4}\Sigma H_i = 0$

If the simple hypothesis that $H = H_1 = H_2 = H_3 = H_4$ is made (and $B_i = \frac{1}{4}\Sigma B_i$, $L_i = \frac{1}{4}\Sigma L_i$)

then

$$\Delta A_i = \Delta A = H - \tfrac{1}{4}(4H) = 3/4\ H$$

that is

all ΔA_i are equal and are equal to $3/4\ H$

If the simple hypothesis $H_1 = H_3$, $H_2 = H_4$, $H_1 = -H_2$ (and $B_i = \frac{1}{4}\Sigma B_i$, $L_i = \frac{1}{4}\Sigma L_1$) (which could be inferred from Brown's work)

then

$$\Sigma H_i = 0$$

and indeed

$$\Delta A_i = H_i$$

and

$$\Delta A_i = H_1 = H_3 = H_2 = - H_4$$

Considering now condition 2) $\quad B_i - \frac{1}{4}\Sigma B_i = 0$

Because of the experimental conditions it is most unlikely that any bias due to the angle of the light pipe, assymetry of the grid, etc. would lead to a condition where the B_i's were not all equal in magnitude and direction. If this true, i.e., $B_1 = B_2 = B_3 = B_4$

then

$$B_i - \tfrac{1}{4}\ (4B_i) = 0$$

and the desired condition is achieved.

Considering condition 3) $\quad L_i - \frac{1}{4}\Sigma L_i = 0$

Several possible circumstances will be considered

a) The lunar effect is the same on a worm regardless of its initial direction of travel, i.e., $L_1 = L_2 = L_3 = L_4$ in this case the lunar effect is entirely removed in ΔA_i since

$$L_i - \frac{1}{4}(4L_i) = 0$$

b) Similarly this will be true for the trivial case where

$$L_1 = L_2 = L_3 = L_4 = 0$$

c) For the case where $\Sigma L_i = 0$ we do not get a removal of the lunar effect.

It is considered likely that the condition (a) is approximated and that the lunar effect is completely or at least partially removed by forming the difference angle $(A_i - \bar{A})$ which in the notation used in the body of the report corresponds to the $(Al\text{-}Al_{avg})$ and $(Mu\text{-}Mu_{avg})$.

In the case of the analysis where the (Al-Mu) angle is used as the criterion measure, we have the following condition for the removal of any bias or lunar effects.

A = Al = total angle for non-shielded case

M = Mu = total angle for the shielded case

H = component due to magnetic field (H = 0 in the shield)

B = bias component due to apparatus

L = lunar component

Now assuming no interaction between the three components, it is considered very unlikely that the B and L components will be different in the Al and Mu cases.

We then have

$$A_i = H_i + B_i + L_i$$

$$M_i = 0 + B_i + L_i$$

$$(A_i - M_i) = (Al\text{-}Mu) = (H_i + B_i + L_i) - (0 + B_i + L_i)$$

$$(Al - Mu) = H_i$$

This is the desired condition, that the bias and lunar effects are completely removed.

If H_i for Mu is not 0 but is a reduced value H_i', then we have

$(Al\text{-}Mu) = H_i - H_i'$ purely a magnetic component

On the basis of the above analysis, it is felt that this difference angle is superior to difference angles formed by $(Al\text{-}Al_{avg})$ or $(Mu\text{-}Mu_{avg})$ in revealing a magnetic component due only to a magnetic stimulus

TABLE A

The Mean Angle (of 18 Worms) under Earth's Field Condition for Each Direction and Each Experiment Day with Standard Error of Mean Angle

Exp.	$A1_N$	$A1_E$	$A1_S$	$A1_W$	$SE(A1_N)$	$SE(A1_E)$	$SE(A1_S)$	$SE(A1_W)$
13	-2.000	+3.389	+1.167	-4.667	2.994	2.663	4.380	3.409
14	+0.389	+5.333	+8.056	+2.056	2.539	1.858	2.964	2.603
15	+0.167	-5.167	+2.056	-1.278	2.012	2.130	1.767	2.834
16	+12.133	+7.000	+2.933	+11.400	4.691	5.580	4.901	4.475
17	+4.611	+1.944	+2.000	+3.389	2.679	2.997	4.574	3.985
18	-2.278	-1.444	-2.611	-1.000	4.442	4.232	4.168	3.056
19	-3.944	-6.222	+0.500	-6.833	5.167	5.878	4.621	4.334
20	+3.000	-0.722	+1.389	-2.444	2.649	2.675	1.831	3.063
21	+5.222	+2.000	+5.833	+2.000	3.293	2.791	3.915	4.021
22	+0.167	+8.778	+5.000	-3.667	4.327	3.889	3.740	4.667
23	-1.500	-0.667	+1.000	+0.389	3.276	1.751	3.131	2.627
24	+1.833	+1.333	+5.222	+1.778	2.492	2.305	2.806	2.019
25	+1.500	+1.944	+4.667	-2.500	3.435	4.057	3.814	2.839
26	-2.667	+2.444	+3.667	+2.000	2.501	2.119	2.285	3.411
27	-2.444	+4.278	-0.889	+2.000	5.005	2.097	3.193	3.504
28	-2.500	+4.278	+0.111	-0.111	3.520	4.933	3.729	2.166
29	+3.389	-0.500	+4.833	+6.944	1.870	2.499	2.565	2.629
30	-0.556	-1.389	+3.444	+0.333	3.197	2.356	2.587	3.185
31	+4.444	+10.058	+2.235	+8.111	4.029	3.521	2.820	4.243
32	-4.611	+1.222	+1.389	+1.111	2.238	1.695	2.546	2.463
33	-5.444	-3.278	-1.500	-2.444	2.976	2.903	3.654	2.955
34	-2.333	+2.500	+0.111	+2.000	2.899	2.456	2.122	3.057
35	-1.500	+2.556	+0.111	+1.167	3.915	3.594	3.082	2.685
36	-1.444	-2.389	+1.778	+2.412	4.304	4.436	4.449	3.074

TABLE B

The Mean Angle of 18 Worms under Shielded Condition for Each Direction and Each Experiment Day with Standard Error of Mean Angle

Exp.	Mu_N	Mu_E	Mu_S	Mu_W	$SE(Mu_N)$	$SE(Mu_E)$	$SE(Mu_S)$	$SE(Mu_W)$
13	+1.611	+1.611	-3.333	+0.778	3.101	2.217	2.840	2.843
14	+0.944	-0.722	-0.444	-2.278	2.517	2.852	2.214	2.413
15	-2.667	-0.500	+1.778	+2.333	2.468	1.881	3.038	1.896
16	+9.733	-3.066	-3.000	+11.867	5.872	6.393	6.188	5.168
17	-3.056	+5.111	+6.556	+1.722	2.605	3.873	3.350	4.045
18	+2.278	+6.667	-6.056	-2.500	4.722	5.578	5.028	3.638
19	+0.167	+4.833	+3.833	-14.556	5.582	6.406	4.940	4.751
20	-3.111	+0.611	+0.389	+3.167	3.225	2.825	2.888	2.364
21	+4.778	+13.722	-2.111	-3.278	5.470	4.861	4.479	3.641
22	+2.389	+1.778	+2.889	-6.278	2.881	3.337	3.098	2.696
23	-0.889	+0.056	+1.0001	+0.389	2.770	2.449	2.211	2.008
24	+0.556	+4.111	+2.167	+3.944	2.418	3.094	2.415	2.865
25	-1.778	+5.222	-1.611	+2.667	3.668	2.834	3.068	3.567
26	+2.056	+6.333	+3.833	+7.556	2.349	3.064	1.248	1.995
27	+1.111	+1.667	+5.889	+0.889	4.136	4.616	3.554	3.368
28	+0.0001	+5.444	-1.556	-0.667	3.899	2.346	3.445	2.539
29	+1.278	+1.722	+2.167	+2.556	2.449	2.291	1.681	2.033
30	-1.222	+1.500	+3.278	+0.944	2.877	3.250	3.129	3.216
31	-0.500	+9.889	+3.889	+2.111	3.128	5,166	4.727	3.068
32	-0.333	+3.500	+3.111	+5.278	2.368	2.925	1.580	2.358
33	-3.778	-1.889	-1.5001	-2.111	4.410	4.238	2.430	3.897
34	+2.000	+4.278	+3.167	+2.611	3.489	2.670	2.775	2.388
35	-2.333	+1.778	-3.278	+3.111	3.520	3.813	2.003	4.322
36	-4.444	-1.833	-3.556	+4.667	4.534	3.609	3.521	4.054

TABLE C

The Mean Difference Angle of 18 Worms (Unshielded Minus Shielded Condition) for Each Direction and Each Experiment Day with Standard Error of Mean Angle

Exp.	$(\overline{Al_N - Mu_N})$	$(\overline{Al_E - Mu_E})$	$(\overline{Al_S - Mu_S})$	$(\overline{Al_W - Mu_W})$	$SE(\Delta_N)$	$SE(\Delta_E)$	$SE(\Delta_S)$	$SE(\Delta_W)$
13	-3.611	+1.778	+4.500	-5.444	4.323	2.625	4.458	3.404
14	-0.555	+6.056	+8.500	+4.333	3.170	3.426	3.421	3.837
15	+2.833	-4.667	+0.278	-3.611	2.550	2.328	3.527	3.640
16	+2.389	+4.556	+5.889	-0.444	5.311	5.508	4.584	6.624
17	-1.556	-3.167	-4.556	+1.667	3.663	3.946	4.165	4.020
18	-4.556	-8.111	+3.444	+0.389	5.451	5.973	7.361	4.346
19	-4.111	-3.444	+0.500	+7.722	6.144	5.789	5.668	7.686
20	+6.111	-1.333	+1.000	-5.611	3.884	2.585	3.248	4.516
21	+4.278	-11.722	+7.944	+5.278	3.454	4.997	5.129	4.451
22	-2.222	+7.000	+2.111	+2.611	4.962	3.684	3.552	5.754
23	-0.611	-0.722	+0.000	+0.000	2.938	2.574	3.699	2.519
24	+1.278	-2.778	+3.056	-2.167	3.943	4.619	3.004	3.363
25	+3.278	-3.278	+6.278	-5.167	3.671	4.114	4.589	4.635
26	-4.722	-3.889	-0.167	-5.556	2.898	2.472	2.532	4.079
27	-3.556	+2.611	-6.778	+1.111	5.378	4.729	3.580	2.681
28	-2.500	-1.167	+1.667	+0.556	3.183	5.243	3.641	2.495
29	+2.111	-2.222	+2.667	+4.389	2.323	3.846	3.268	2.558
30	+0.667	-2.889	+0.167	-0.611	4.241	3.137	4.268	4.001
31	+4.944	+3.500	+2.667	+6.000	4.677	7.258	3.287	4.169
32	-4.278	-2.278	-1.722	-4.167	3.351	2.701	2.832	2.857
33	-1.667	+5.167	+0.000	-0.333	5.646	4.971	2.732	4.174
34	-4.333	-1.778	-3.056	-0.611	4.467	3.694	3.327	4.351
35	+0.833	+0.778	+3.389	-1.944	4.890	4.172	2.791	3.792
36	+3.000	-0.556	+5.333	-2.278	4.697	4.765	4.154	4.135

WEIGHTED MEAN
WEIGHT = 1/V

Weighted Mean $\bar{X}_w = \Sigma w_i x_i / \Sigma w_i$ where $w_i = \frac{1}{V_i}$

and $\bar{x}_w/\sqrt{V} = t$ (relative to expected $\bar{x}$ of zero) $\frac{1}{V} = \Sigma \frac{1}{V_i}$

R/0₁ $\bar{X}_w$ = weighted mean
R/0₂ V = variance of $\bar{X}_w$
R/0₃ $\sqrt{V}$ = S.E. $(\bar{X}_w)$
R/0₄ t = $\bar{X}_w$/S.E$(\bar{X}_w)$

Test Program

x_i	V_i
9	5
8	4
7	3
6	2
5	1

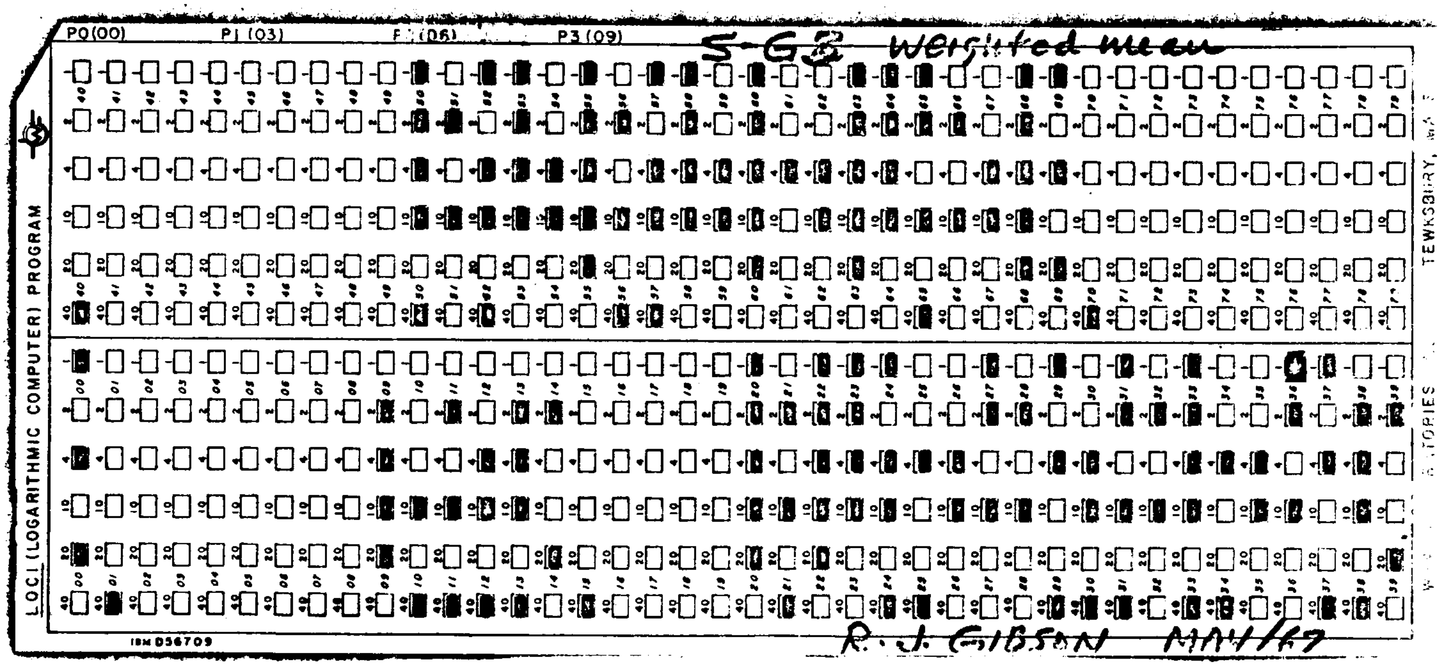
Then: $r/0_1 = 6.1905 = \bar{X}_w$
$r/0_2 = 0.4380 = V(\bar{X}_w)$
$r/0_3 = 0.6618 = \sqrt{V} = S.E(\bar{X}_w)$
$r/0_4 = 9.354 = t = \frac{\bar{X}_w}{S.E(\bar{X}_w)}$

Press P3 — auto display off (UP)
Enter x_i - run
Enter V_i - run (note never enter $V_i = 0$)
repeat for all x_i's & V_i's
Press P0 get r/0₁
run get r/0₂
run get r/0₃
run get r/0₄
run get r/0₁ or press P3 to enter new data
repeats readouts

No.	Cmd	Code	Comment	No.	Cmd	Code	Comment	No.	Cmd	Code	Comment	No.	Cmd	Code	Comment
00	5	25	5 in W	20	Stop	37	Enter X_i - run	40	W→PC	40	go to 20	60	Stop	37	r/0₂ V (run)
1	W→PC	40	go to 50	21	W→S₁	52	temp store X_i	~~41~~	Press	P₁ for	R/0's	61	√	24	√V in L
2				22	Stop	37	Enter V_i - run	42	otherwise continue to			62	LN⁻¹	14	√V in W = [S.E($\bar{X}_w$)]
3				23	÷	17	$1/V_i$ in L	43	enter new		x_i's & V_i's	63	Stop	37	r/0₃ √V (run)
4				24	S₂→W	55	old Σ1/V_i →W	44				64	÷	17	$1/\sqrt{V}$ in L
05				25	W→A	44	" " →A	45				65	S₁→W	53	$\bar{X}_w$ → W
6				26	LN⁻¹	14	$1/V_i$ → W	46				66	×	12	$\bar{X}_w/\sqrt{V}$ = t in L
7				27	+	13	$1/V_i$ + old $\Sigma 1/V_i$	47				67	LN⁻¹	14	$\bar{X}_w/\sqrt{V}$ = t →W
8				28	×	12	$1/V_i$ into L	48				68	Stop	37	r/0₄ t in W (run)
9	Prime	36		29	A→W	45	New $\Sigma 1/V_i$ →W	49				69	5	25	5 in W
10	W→S0	50		30	W→S₂	54	New $\Sigma 1/V_i$ → S₂	50	S₃→W	57	New $\Sigma \frac{X_i}{V_i}$ → W	70	W→PC	40	go to 50
11	W→S₁	52		31	S₁→W	53	x_i → W	51	×	12	" → L	71	otherwise For new	set of	data Press P₃
12	W→S₂	54		32	×	12	x_i/V_i in L	52	S₂→W	55	New $\Sigma(\frac{1}{V_i})$ → W	72			
13	W→S₃	56		33	S₃→W	57	old $\Sigma \frac{X_i}{V_i}$ → W	53	÷	17	$\Sigma x_i/V_i / \Sigma(\frac{1}{V_i})$ in L	73			
14	2	22		34	W→A	44	" " A	54	LN⁻¹	14	$\bar{X}_w$ in W	74			
15	W→PC	40	go to 20	35	LN⁻¹	14	x_i/V_i → W	55	Stop	37	r/0₁ $\bar{X}_w$ - run	75			
16				36	+	13	$x_i/V_i + \Sigma \frac{x_i}{V_i}$	56	W→S₁	52	Store $\bar{X}_w$ in S₁	76			
17				37	A→W	45	New $\Sigma x_i/V_i$ → W	57	S₂→W	55	$\Sigma(\frac{1}{V_i})$ in W	77			
18				38	W→S₃	56	" " → S₃	58	÷	17	$1/\Sigma(\frac{1}{V_i})$ in L	78			
19				39	2	22	2 into W	59	LN⁻¹	14	$1/\Sigma(\frac{1}{V_i})$ = V → W	79			

(run)
To read r/0's again press run after 68 (stop)

WEIGHTED MEAN, WEIGHT = 1/v



LOCI Program for use in ANOVA — Sums, squares & Sums of squares — Date: Feb/67 — RG

SUM, SQUARE, SUMS OF SQS

Sums, squares, s^2
Sums of squares for ANOVA

P_0 - to start program

Put in x_i's press run

at end of column press P_1

$r/o_1 = \Sigma x_i$ for column press run

$r/o_2 = \Sigma x_i^2$ for column press run

r/o_3 Σx_i grand previous & present columns - press run

r/o_4 Σx_i^2 " " " " " - press run

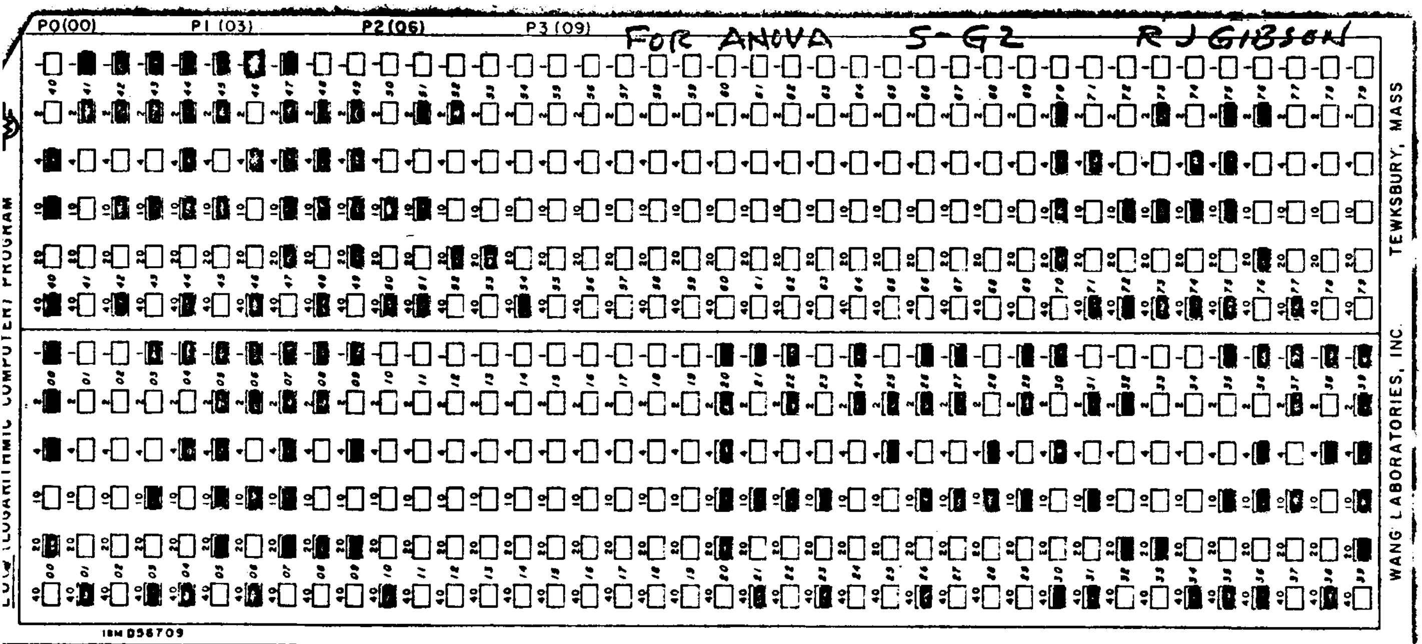
Test numbers:

$x_1 = 1$	$x_1' = 2$	$r/o_1 = 2$	$r/o_1' = 3$
$x_2 = -2$	$x_2' = -3$	$r/o_2 = 14$	$r/o_2' = 29$
$x_3 = 3$	$x_3' = 4$	$r/o_3 = 2$	$r/o_3' = 2+3 = 5$
		$r/o_4 = 14$	$r/o_4' = 14+29 = 43$

Press P_1 for readouts

No.	Cmd	Code	Comment	No.	Cmd	Code	Comment	No.	Cmd	Code	Comment	No.	Cmd	Code	Comment
00	7	27		20	Stop	37	put in x_i press run	40	$W \to S_2$	54	store new grand Σx_i	60			
1	$W \to PC$	40	goto 70 for	21	$S_0 \to A$	51	part of colm tot	41	clear A	03		61			
2			clean up	22	+	13	add	42	$S_1 \to W$	53	colmn tot x_i^2 retrieve	62			
3	$S_0 \to A$	51		23	$A \to S_0$	50	new colm total stored	43	+	13	add	63			
4	$A \to W$	45		24	clear A	03		44	$S_3 \to W$	57	old grand Σx_i^2 retrieve	64			
05	Stop	37	read out colmn Σx_i - run	25	$\square$	06	sq x_i	45	+	13	add	65			
6	$S_1 \to W$	53		26	$S_1 \to W$	53	part of colm tot	46	$A \to W$	45	display new grand Σx_i^2	66			
7	stop	37	readout colmn Σx_i^2 - run	27	+	13	add	47	Stop	37	r/o Σx_i^2 grand run	67			
8	3	23		28	LN^{-1}	14	x_i^2 into W	48	$W \to S_3$	56	store Σx_i^2 grand new	68			
9	5	25		29	+	13	add	49	Prime	36	clear A, W, L	69			
10	$W \to PC$	40	go to 35 for	30	$A \to W$	45		50	$A \to S_0$	50	clear colm tot	70	Prime	36	clears W, A, L
11			Grand readouts	31	$W \to S_1$	52	store new column total	51	$W \to S_1$	52	clear colmn tot	71	$W \to A$	44	
12				32	2	22		52	2	22		72	$A \to S_0$	50	
13				33	0	20		53	0	20		73	$W \to S_1$	52	
14				34	$W \to PC$	40	go back to 20	54	$W \to PC$	40	return to 20	74	$W \to S_2$	54	
15				35	$S_0 \to A$	51	colmn tot x_i	55				75	$W \to S_3$	56	
16				36	$S_2 \to W$	55	old grand tot x_i	56				76	2	22	
17				37	+	13	new grand Σx_i	57				77	$W \to PC$	40	go to 20
18				38	$A \to W$	45	display	58				78			
19				39	Stop	37	r/o [illegible]	59				79			

SUMS, SQUARES, SUMS OF SQUARES



MEAN, STANDARD DEVIATION & STANDARD ERROR OF MEAN

Modified S-105A MOD I-A
Statistical

Σx in S_0
Σx^2 in S_1
n in S_2

① Start P_3
② Enter x hit run
Continue
③ P_1 r/0_1 = n, run
④ r/0_2 = $\bar{x}$, run
⑤ r/0_3 = Sum of Sq's SS $\Sigma(x^2) - \frac{(\Sigma x)^2}{n}$, run

⑥ r/0_4 SS/d.f = S_x^2
⑦ r/0_5 S_x
⑧ r/0_6 $S_{\bar{x}}$ = S.E.($\bar{x}$)
⑨ r/0_7 S.E^2($\bar{x}$)

No.	Cmd	Code	Comment	No.	Cmd	Code	Comment	No.	Cmd	Code	Comment	No.	Cmd	Code	Comment
00				20	A→W	45		40	A→W	45	n	60	W→A	44	n
1				21	W→S_1	52		41	Stop	37		61	1	21	$n-1$
2				22	S_0→A	51		42	W→S_2	54	n→S_2	62	−	15	
3	3	23	P_1	23	Stop	37	enter x	43	÷	17	$1/n$	63	A→W	45	
4	5	25		24	S_0→A	51		44	S_0→A	51	$\Sigma(x)$ in A	64	÷	17	
05	W-Pc	40		25	+	13	add x to prev. x	45	A→W	45	$\Sigma(x)$ in W	65	LN^{-1}	14	$SS/n-1$
6				26	A→S_0	50	store	46	×	12		66	Stop	37	S_x^2
7				27	□	6	square x	47	LN^{-1}	14	$\bar{x}$	67	$\sqrt{\ }$	4	
8				28	TEST DC=0	70		48	Stop	37		68	LN^{-1}	14	S_x
9	Prime	36	P_3	29	1	21		49	□	6	$\bar{x}^2$	69	Stop	37	
10	W-DC	42	DC=100=00	30	5	25		50	S_2→W	55		70	×	12	
11	A-S_0	50		31	W→PC	40	if ≠ 0 jump to 5	51	×	12		71	S_2→W	55	n
12	2	22		32	Stop	37	stop if DC=0	52	S_1→W	53	Σx^2	72	$1/\sqrt{\ }$	5	
13	Dec DC	66		33				53	W→A	44		73	LN^{-1}	14	$S_{\bar{x}}$ = S.E
14	W→Pc	40		34				54	LN^{-1}	14	$n\bar{x}^2$	74	Stop	37	
15	Dec DC	66		35	9	31		55	−	15		75	□	6	
16	S_1→W	53		36	9	31		56	A→W	45	SS	76	LN^{-1}	14	$S_{\bar{x}}^2$ = S.E^2
17	W→A	44		37	W→A	44		57	Stop	37		77	Stop	37	
18	LN^{-1}	14		38	DC→W	43		58	×	12		78			
19	+	13		39	−	15		59	S_2→W	55	n	79			

MEAN, STANDARD DEVIATION & STANDARD ERROR OF MEAN